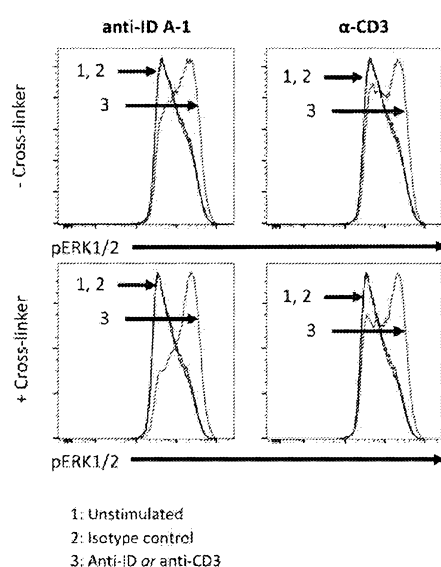




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- (71) **Applicant:** JUNO THERAPEUTICS, INC. [US/US]; 400 Dexter Ave. N, Suite 1200, Seattle, WA 98109 (US).
- (72) **Inventors:** HAUSKINS, Collin; 400 Dexter Ave. N, Suite 1200, Seattle, WA 98109 (US). HEIPEL, Mark, D.; 400 Dexter Ave. N, Suite 1200, Seattle, WA 98109 (US). SUTHERLAND, Claire, L.; 400 Dexter Ave. N, Suite 1200, Seattle, WA 98109 (US). SATHALIYA, Taher; 400 Dexter Ave. N, Suite 1200, Seattle, WA 98109 (US). SMITH, Jeff; 400 Dexter Ave. N, Suite 1200, Seattle, WA 98109 (US).
- (74) **Agent:** POTTER, Karen et al.; Morrison & Foerster LLP, 12531 High Bluff Drive, Suite 100, San Diego, CA 92130-2040 (US).
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(54) **Title:** ANTI-IDIOTYPIC ANTIBODIES AGAINST ANTI-CD19 ANTIBODIES**FIG. 1**

(57) **Abstract:** Provided herein are anti-idiotype antibodies that specifically recognize anti-CD19 antibody moieties, in particular, anti-CD19 antibody moieties present in recombinant receptors, including chimeric antigen receptors (CARs). The disclosure further relates to uses of anti-idiotype antibodies for specifically identifying and/or selecting cells expressing such recombinant receptors, such as anti-CD19 CAR T cells. The disclosure further relates to uses of anti-idiotype antibodies for specifically activating such cells.

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ANTI-IDIOTYPIC ANTIBODIES AND RELATED METHODS

Cross-Reference to Related Applications

[0001] The application claims the benefit of priority to U.S. provisional patent application 62/369,008, filed July 29, 2016, entitled “ANTIBODIES AND RELATED METHODS,” the contents of which is hereby incorporated by reference in their entirety for all purposes.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042006540SeqList.TXT, created July 15, 2017 which is 86,050 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates in some aspects to anti-idiotypic antibodies that specifically recognize anti-CD19 antibody moieties, in particular, anti-CD19 antibody moieties present in recombinant receptors, including chimeric antigen receptors (CARs). The disclosure further relates to uses of anti-idiotypic antibodies for specifically identifying or selecting cells expressing such recombinant receptors, such as anti-CD19 CAR T cells. The disclosure further relates to uses of anti-idiotypic antibodies for specifically activating such cells.

Background

[0004] Methods are available for adoptive cell therapy using engineered cells expressing recombinant receptors, such as chimeric antigen receptor (CARs) containing extracellular antibody antigen-binding domains. Various strategies are available to assess activity of such cells either *in vitro* or upon *in vivo* to a subject. Improved methods are needed to specifically assess activity of CAR-expressing cells. Provided are reagents, compositions, and articles of manufacture that meet such needs.

Summary

[0005] Provided herein are agents that specifically bind to antibodies, including antibody fragments such as scFvs, and chimeric molecules containing the same, such as chimeric antigen receptors. Also provided are compositions and articles of manufacture containing such agents, including those including a surface to which the agent is bound, such as a solid surface, e.g. a plate or bead. Also among the embodiments provided herein are uses and methods of using such agents, compositions and articles, including for detection, use, manipulation and/or stimulation of cells or therapies containing or suspected of containing the antibody or chimeric molecule, such as in the detection, stimulation or use of CAR-expressing cells.

[0006] In some aspects, the antibody is or includes an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is or contains variable region(s) of the antibody designated SJ25C1, and/or an antigen-binding fragment thereof. In some embodiments, the agent, e.g., the anti-idiotypic antibody or antigen-binding fragment, contains a light chain variable (VL) region containing at least 90% sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VL region amino acid sequence set forth in SEQ ID NO: 5; and/or a heavy chain variable (VH) region containing at least 90% sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VH region amino acid sequence set forth in SEQ ID NO: 1.

[0007] Provided herein is an antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment contains a VL region containing at least 90% sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VL region amino acid sequence set forth in SEQ ID NO: 5; and/or a VH region containing at least 90 % sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VH region amino acid sequence set forth in SEQ ID NO: 1.

[0008] In some of any such embodiments, the VH region contains a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence set forth in SEQ ID NO: 11 or 84 or containing a CDR-H3 contained within the VH sequence set forth in SEQ ID NO: 1; and/or the VL region contains a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence set forth in SEQ ID NO: 14 or 87 or containing a CDR-L3 contained within the VL sequence set forth in SEQ ID NO: 5.

[0009] In some of any such embodiments, the VH region contains a CDR-H1 and a CDR-H2, respectively, containing the amino acid sequences of CDR-H1 and CDR-H2 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 1; and/or the VL region contains a CDR-L1 and CDR-L2, respectively, containing the amino acid sequences of CDR-L1 and CDR-L2 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 5.

[0010] In some of any such embodiments, the VH region contains a CDR-H1 set forth in SEQ ID NO: 9, 78, 79, or 80, a CDR-H2 set forth in SEQ ID NO: 10, 81, 82, or 83, and a CDR-H3 set forth in SEQ ID NO: 11 or 84; and/or the VL region contains a CDR-L1 set forth in SEQ ID NO: 12 or 85, a CDR-L2 set forth in SEQ ID NO: 13 or 86, and a CDR-L3 set forth in SEQ ID NO: 14 or 87.

[0011] Provided herein is an anti-idiotypic antibody or antigen-binding fragment thereof containing a CDR-H1, a CDR-H2, and a CDR-H3, respectively, containing the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 1; and/or a CDR-L1, a CDR-L2, and a CDR-L3, respectively, containing the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 5.

[0012] Provided herein is an anti-idiotypic antibody or antigen-binding fragment thereof containing a CDR-H1 containing the amino acid sequence of SEQ ID NO: 9, 78, 79, or 80, a CDR-H2 containing the amino acid sequence of SEQ ID NO: 10, 81, 82 or 83, and a CDR-H3 containing the amino acid sequence set forth as SEQ ID NO: 11 or 84; and/or a CDR-L1 containing the amino acid sequence of SEQ ID NO: 12 or 85, a CDR-L2 containing the amino acid sequence of SEQ ID NO: 13 or 86, and a CDR-L3 containing the amino acid sequence of SEQ ID NO: 14 or 87.

[0013] In some of any such embodiments, the VH region of the antibody or fragment contains the amino acid sequence of SEQ ID NO: 1; and/or the VL region of the antibody or fragment contains the amino acid sequence of SEQ ID NO: 5. In some embodiments, the VH region of the antibody or fragment contains the amino acid sequence of SEQ ID NO: 1 and the VL region of the antibody or fragment contains the amino acid sequence of SEQ ID NO: 5. In some of any such embodiments, the target antibody or antigen-binding fragment contains a

heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

[0014] In some embodiments, the agent is an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is or contains variable region(s) of the antibody designated FMC63 or an antigen-binding fragment thereof and/or specifically binds to a chimeric molecule containing such an antibody fragment, such as a CAR with a binding domain containing antibody variable regions or portions thereof derived from FMC63, such as in the form of an scFv. In some embodiments, the agent, e.g., the anti-idiotypic antibody or antigen-binding fragment contains a light chain variable (VL) region containing at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62; and/or a heavy chain variable (VH) region containing at least 90% sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58. In some embodiments, the antibody or antigen-binding fragment contains a VL region comprising at least 90% sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62; and/or a VH region comprising at least 90 % sequence identity (and/or at least 95 % or 99 % sequence identity, or 100 % identity) to the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58.

[0015] In some of any such embodiments, the VH region contains a heavy chain complementarity determining region 1 (CDR-H1) including the amino acid sequence of GYX₃FX₅X₆YX₈MX₁₀ (SEQ ID NO: 108), wherein X₃ is T or S, X₅ is T or S, X₆ is D or R, X₈ is Y or W, and X₁₀ is K or N; a heavy chain complementarity determining region 2 (CDR-H2) including the amino acid sequence WIGX₄IX₆PX₈X₉X₁₀X₁₁TX₁₃X₁₄NQX₁₇FKX₂₀ (SEQ ID NO: 109), wherein X₄ is D or M, X₆ is N or H, X₈ is N or S, X₉ is N or D, X₁₀ is G or S, X₁₁ is G or E, X₁₃ is D or R, X₁₄ is Y or L, X₁₇ is N or K, and X₂₀ is G or D; a heavy chain complementarity determining region 3 (CDR-H3) including the amino acid sequence AX₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅ (SEQ ID NO: 110), wherein X₂ is R or S, X₃ is E or I, X₄ is G or Y, X₅ is N or Y, X₆ is N or E, X₇ is Y or null, X₈ is G or null, X₉ is S or null, X₁₀ is R or null, X₁₁ is D or null, X₁₂ is A or null, X₁₃ is M or null, X₁₄ is D or E, and X₁₅ is Y or A; and/or the VL region contains a light chain complementarity determining region 3 (CDR-L1) including

the amino acid sequence $X_1AX_3X_4X_5X_6X_7X_8YX_{10}X_{11}WY$ (SEQ ID NO: 111), wherein X_1 is S or R, X_3 is S or R, X_4 is S or G, X_5 is G or N, X_6 is V or I, X_7 is I or H, X_8 is N or null, X_{10} is M or L, and X_{11} is Y or A; a light chain complementarity determining region 2 (CDR-L2) including the amino acid sequence $X_1X_2X_3YX_5X_6X_7X_8LAX_{11}$ (SEQ ID NO: 112), wherein X_1 is P or L, X_2 is W or L, X_3 is I or V, X_5 is L or N, X_6 is T or A, X_7 is S or K, X_8 is N or T, and X_{11} is S or D; a light chain complementarity determining region 3 (CDR-L3) including the amino acid sequence $QX_2X_3X_4X_5X_6PX_8T$ (SEQ ID NO: 113), wherein X_2 is Q or H, X_3 is W or F, X_4 is S or W, X_5 is S or W, X_6 is N or T, and X_8 is L or Y.

[0016] In some embodiments, the complementarity determining region 3 (CDR-H3) contains the amino acid sequence set forth in SEQ ID NO: 94 or 104 or contains a CDR-H3 contained within the VH sequence set forth in SEQ ID NO: 36 or 58; and/or the light chain complementarity determining region 3 (CDR-L3) contains the amino acid sequence set forth in SEQ ID NO: 97 or 107 or containing a CDR-L3 contained within the VL sequence set forth in SEQ ID NO: 40 or 62.

[0017] In some embodiments, the VH region contains a CDR-H1 and a CDR-H2, respectively, including the amino acid sequences of CDR-H1 and CDR-H2 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or the VL region contains a CDR-L1 and CDR-L2, respectively, including the amino acid sequences of CDR-L1 and CDR-L2 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0018] In some of any such embodiments, the VH region contains a CDR-H1 set forth in SEQ ID NO: 88, 89, 90, 98, 99, or 100, a CDR-H2 set forth in SEQ ID NO: 91, 92, 93, 101, 102, or 103 and a CDR-H3 set forth in SEQ ID NO: 94 or 104; and/or the VL region contains a CDR-L1 set forth in SEQ ID NO: 95 or 105, a CDR-L2 set forth in SEQ ID NO: 96 or 106, and a CDR-L3 set forth in SEQ ID NO: 97 or 107.

[0019] Provided herein is an anti-idiotypic antibody or antigen-binding fragment thereof containing a CDR-H1, a CDR-H2, and a CDR-H3, respectively, containing the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or a CDR-L1, a CDR-L2, and a CDR-L3, respectively, including the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3

sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0020] Provided herein is an anti-idiotypic antibody or antigen-binding fragment thereof comprising a CDR-H1 including the amino acid sequence of SEQ ID NO: 88, 89, 90, 98, 99, or 100, a CDR-H2 including the amino acid sequence of SEQ ID NO: 91, 92, 93, 101, 102, or 103, and a CDR-H3 including the amino acid sequence set forth as SEQ ID NO: 94 or 104; and/or a CDR-L1 including the amino acid sequence of SEQ ID NO: 95 or 105, a CDR-L2 including the amino acid sequence of SEQ ID NO: 96 or 106, and a CDR-L3 including the amino acid sequence of SEQ ID NO: 97 or 107.

[0021] In some of any such embodiments, the VH region of the antibody or fragment contains the amino acid sequence of SEQ ID NO: 36 or 58; and/or the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40 or 62. In some instances, the VH region of the antibody or fragment includes the amino acid sequence of SEQ ID NO: 36 or 58 and the VL region of the antibody or fragment includes the amino acid sequence of SEQ ID NO: 40 or 62.

[0022] In some of any such embodiments, the VH region contains a CDR-H1 set forth in SEQ ID NO: 44, 88, 89, or 90, a CDR-H2 set forth in SEQ ID NO: 45, 91, 92, or 93 and a CDR-H3 set forth in SEQ ID NO: 46 or 94; and/or the VL region contains a CDR-L1 set forth in SEQ ID NO: 47 or 95, a CDR-L2 set forth in SEQ ID NO: 48 or 96, and a CDR-L3 set forth in SEQ ID NO: 49 or 97. In some of any such embodiments, the VH region contains a CDR-H1 set forth in SEQ ID NO: 65, 98, 99, or 100, a CDR-H2 set forth in SEQ ID NO: 66, 101, 102, or 103 and a CDR-H3 set forth in SEQ ID NO: 67 or 104; and/or the VL region contains a CDR-L1 set forth in SEQ ID NO: 68 or 105, a CDR-L2 set forth in SEQ ID NO: 69 or 106, and a CDR-L3 set forth in SEQ ID NO: 100 or 107.

[0023] In some of any such embodiments, the VH region contains a CDR-H1, CDR-H2 and CDR-H3 including the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36; and/or the VL region contains a CDR-L1, a CDR-L2, and a CDR-L3, respectively, including the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40.

[0024] In some of any such embodiments, the VH region contains a CDR-H1, CDR-H2 and CDR-H3 including the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 58; and/or the VL region contains a CDR-L1, a CDR-L2, and a CDR-L3, respectively, including the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 62.

[0025] In some of any such embodiments, the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36; and/or the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40. In some of any such embodiments, the VH region of the antibody or fragment includes the amino acid sequence of SEQ ID NO: 58; and/or the VL region of the antibody or fragment includes the amino acid sequence of SEQ ID NO: 62.

[0026] In some of any such embodiments, the target antibody or antigen-binding fragment is a single chain fragment. In some aspects, the fragment contains antibody variable regions joined by a flexible linker. In some of any such embodiments, the fragment contains an scFv.

[0027] In some of any such embodiments, the target antibody or antigen-binding fragment contains a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24; and/or is an scFv comprising the sequence of amino acids set forth in SEQ ID NO: 28. In some embodiments, the target antibody or antigen-binding fragment contains a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31; and/or is an scFv comprising the sequence of amino acids set forth in SEQ ID NO: 34.

[0028] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment specifically binds to the same or an overlapping epitope of a target antibody or antigen-binding fragment thereof as the epitope specifically bound by the anti-idiotypic antibody or antigen-binding fragment according to any one of the embodiments described herein.

[0029] In some of any such embodiments, the target antibody or antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR); and/or the anti-idiotypic antibody or antigen-binding fragment specifically binds the target antibody or antigen-binding fragment contained within or included

in the antigen-binding domain of the extracellular portion of a CAR. In some embodiments, the target antibody or antigen-binding fragment is an scFv and the anti-idiotypic antibody or antigen-binding fragment specifically binds to an epitope in the scFv of the CAR.

[0030] In some of any such embodiments, the antibody or fragment specifically binds to a single chain variable fragment (scFv) derived from antibody SJ25C1 contained in the extracellular portion of a chimeric antigen receptor, optionally wherein the scFv derived from antibody SJ25C1 contains a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24; and/or contains the sequence of amino acids set forth in SEQ ID NO: 28. In some of any such embodiments, the antibody or fragment specifically binds to a single chain variable fragment (scFv) derived from antibody FMC63 comprised in the extracellular portion of a chimeric antigen receptor, optionally wherein the scFv derived from antibody FMC63 contains a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31; and/or contains the sequence of amino acids set forth in SEQ ID NO: 34.

[0031] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment specifically binds to an epitope within or including all or a portion of a complementarity determining region (CDR) of the target antibody or antigen-binding fragment.

[0032] In some of any such embodiments, the CAR further contains a transmembrane domain linked to the antigen-binding domain via a spacer. In some embodiments, the spacer contains an extracellular portion from CD28, which optionally is human CD28. In some aspects, the extracellular portion from CD28 contains the sequence of amino acids set forth in SEQ ID NO: 27. In some of any such embodiments, the transmembrane domain contains a transmembrane portion of CD28, which optionally is human CD28. In some of any such embodiments, the antibody or fragment does not bind to an epitope in the spacer domain of the CAR.

[0033] In some of any such embodiments, the antibody or fragment does not bind or does not specifically bind to CD28 or a portion thereof, which optionally is human CD28, which optionally contains an extracellular portion of CD28, which optionally contains the sequence of amino acids set forth in SEQ ID NO: 27. In some of any such embodiments, the antibody or

fragment does not bind to an epitope in an Fc domain, which optionally is a human IgG1 Fc domain.

[0034] In some of any such embodiments, the target antibody or antigen-binding fragment specifically binds to human CD19. In some of any such embodiments, the anti-idiotypic antibody or fragment does not cross-react with another anti-CD19 antibody, which optionally is contained in the extracellular antigen-binding domain of another CAR. In some of any such embodiments, the anti-idiotypic antibody or fragment does not cross-react with another CAR.

[0035] In some of any such embodiments, the anti-idiotypic antibody or fragment is an agonist antibody of a CAR containing the target antibody or antigen-binding fragment. In some of any such embodiments, the antibody or fragment is an antagonist of a CAR containing the target antibody or antigen-binding fragment.

[0036] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is humanized. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is recombinant. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is monoclonal.

[0037] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is an antigen-binding fragment. In some aspects, the antigen-binding fragment is selected from among fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, a single chain variable fragment (scFv) or a single domain antibody.

[0038] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof contains at least a portion of an immunoglobulin constant region. In some embodiments, the at least a portion of an immunoglobulin constant region contains an Fc region or a portion of the Fc containing the CH2 and CH3 domains. In some aspects, the constant region is derived from human IgG. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment is an intact antibody or full-length antibody.

[0039] In some of any such embodiments, provided is a conjugate containing the anti-idiotypic antibody or antigen-binding fragment according to any one of the embodiments described above and a heterologous molecule or moiety. In some embodiments, the heterologous molecule or moiety is a label. In some aspects, the label is selected from a fluorescent dye, a fluorescent protein, a radioisotope, a chromophore, a metal ion, a gold particle, a silver particle,

a magnetic particle, a polypeptide, an enzyme, a streptavidin, a biotin, a luminescent compound or an oligonucleotide. In some instances, the heterologous molecule or moiety is a protein, peptide, nucleic acid or small molecule, which optionally is or contains a toxin, Strep-Tag.

[0040] In some embodiments, provided is a nucleic acid molecule(s) encoding the heavy chain and/or light chain of the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described herein. In some aspects, the nucleic acid molecule contains a sequence of nucleotides encoding (i) the heavy chain variable region set forth in SEQ ID NO: 15, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 15; or (iii) a degenerate sequence of (i) or (ii); and/or a sequence of nucleotides encoding (iv) the light chain variable region set forth in SEQ ID NO: 19, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 19; or (vi) a degenerate sequence of (iv) or (v).

[0041] In some of any such embodiments, the nucleic acid molecule contains a sequence of nucleotides encoding (i) the heavy chain set forth in SEQ ID NO: 17, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 17; or (iii) a degenerate sequence of (i) or (ii); and/or a sequence of nucleotides encoding (iv) the light chain set forth in SEQ ID NO: 21, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 21; or (vi) a degenerate sequence of (iv) or (v).

[0042] In some embodiments, the nucleic acid molecule contains a sequence of nucleotides encoding (i) the heavy chain variable region set forth in SEQ ID NO: 50 or 71, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 50 or 71; or (iii) a degenerate sequence of (i) or (ii); and/or a sequence of nucleotides encoding (iv) the light chain variable region set forth in SEQ ID NO: 54 or 75, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 54 or 75; or (vi) a degenerate sequence of (iv) or (v). In some embodiments, the nucleic acid molecule contains a sequence of nucleotides encoding (i) the heavy chain set forth in SEQ ID NO: 52 or 73, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 52 or 73; or (iii) a degenerate sequence of (i) or (ii); and/or a sequence of nucleotides encoding (iv) the light chain

set forth in SEQ ID NO: 56 or 76, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 56 or 76; or (vi) a degenerate sequence of (iv) or (v). In some embodiments, the nucleotide sequence encoding the heavy chain and/or light chain contains a signal sequence.

[0043] Provided herein is a vector containing the nucleic acid molecule according to any one of the embodiments described herein. Also provided herein is a cell containing the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described herein or the nucleic acid molecule according to any one of the embodiments described herein.

[0044] Provided herein is a method of producing an anti-idiotypic antibody or antigen-binding fragment thereof, including expressing the heavy and/or light chain encoded by the nucleic acid molecule according to any one of the embodiments described herein or the vector according to any one of the embodiments described herein in a suitable host cell and recovering or isolating the antibody. In some embodiments, the method of producing an anti-idiotypic antibody or antigen-binding fragment includes culturing the cell according to any one of the embodiments described herein under conditions in which the heavy chain and/or light chain is expressed and recovering or isolating the antibody. Also provided herein is an anti-idiotypic antibody or antigen-binding fragment thereof produced by the method according to any one of the embodiments described herein.

[0045] In some embodiments, provided is a composition containing the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described herein, the conjugate according to any one of the embodiments described herein, or the cell according to any one of the embodiments described herein. In some of any such embodiments, the composition further contains a pharmaceutically acceptable excipient.

[0046] In some embodiments, provided is a kit containing one or more of the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described herein, the conjugate according to any one of the embodiments described herein, the nucleic acid according to any one of the embodiments described herein, and, optionally, instructions for use. In some instances, the kit further contains a reagent or support for immobilizing the anti-idiotypic antibody or antigen-binding fragment thereof or conjugate,

wherein said reagent or support is a bead, a column, a microwell, a stick, a filter, a strip or a soluble oligomeric streptavidin mutein reagent.

[0047] Also provided are methods of detection using any of the provided agents, such as the anti-idiotypic antibodies. In some embodiments, provided is a method of detecting a target antibody or antigen-binding fragment thereof, such as a CAR containing the same, including (a) contacting a composition containing a target antibody (such as one with variable regions derived from an antibody SJ25C1 or from an antibody FMC63, or from an antigen-binding fragment of either of such antibodies) with the anti-idiotypic antibody or antigen-binding fragment thereof; and (b) detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment and/or detecting the presence or absence of the target antibody or agent.

[0048] In some embodiments, the method includes (a) contacting a composition containing or suspected of containing a target antibody that is the antibody FMC63 or an antigen-binding fragment with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of the embodiments described or the conjugate of any of the embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and (b) detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment and/or detecting the presence or absence of the target antibody or agent. .

[0049] In some aspects, the target antibody or antigen-binding fragment is bound to a cell or expressed on the surface of a cell and detecting in (b) includes detecting cells bound with the anti-idiotypic antibody. In some instances, the cell expresses on its surface a CAR containing the target antibody or target antigen-binding fragment.

[0050] In some embodiments, the provided methods involve detecting a CAR containing a target antibody or antigen-binding fragment thereof of any of the embodiments, such as a CAR containing variable domains derived from FMC63 or SJ25C. In some aspects, the methods include (a) contacting a cell expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described or the conjugate according to any one of the embodiments described that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and (b) detecting cells bound with the anti-idiotypic antibody. In some embodiments, the method

includes (a) contacting a cell expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of the embodiments described or the conjugate of any one of the embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and (b) detecting cells bound with the anti-idiotypic antibody. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is directly or indirectly labeled for detection.

[0051] In some embodiments, provided is a method of selecting cells from a cell population, including (a) contacting a cell population expressing a chimeric antigen receptor (CAR) containing a target antibody or a cell bound to a target antibody with the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described herein or conjugate according to any one of the embodiments described that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, wherein the target antibody is the antibody SJ25C1 or an antigen-binding fragment thereof; and (b) selecting cells bound with the anti-idiotypic antibody. In some embodiments, the method includes (a) contacting a cell population expressing a chimeric antigen receptor (CAR) comprising a target antibody or a cell bound to a target antibody with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of the embodiments described or conjugate of any one of the embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, wherein the target antibody is the antibody FMC63 or an antigen-binding fragment thereof; and (b) selecting cells bound with the anti-idiotypic antibody.

[0052] In some instances, the cells bound with the anti-idiotypic antibody are selected by affinity-based separation. In some aspects, the affinity-based separation is immunoaffinity-based separation. In some of any such embodiments, the affinity-based separation is by flow cytometry. In some embodiments, the affinity-based separation is by magnetic activated cell sorting. In some aspects, the affinity-based separation contains affinity chromatography. In some of any such embodiments, the anti-idiotypic antibody is reversibly bound or immobilized to a support or a stationary phase.

[0053] Also among the provided methods are methods for stimulating cells using the agents, such as stimulating cells containing a molecule such as a CAR that is or contains the target antibody recognized by the anti-idiotypic antibody. In some aspects, the methods involve incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described or the conjugate of any one of the embodiments described that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, thereby generating an output composition containing stimulated cells. In some embodiments, the method includes incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of the embodiments described or the conjugate of any one of the embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, thereby generating an output composition containing stimulated cells.

[0054] In some embodiments, the methods result in proliferation, activation, stimulation, cytokine release, or other functional outcome such as upregulation of an activation marker or cytokine release or production, of cells expressing the chimeric receptor such as the CAR recognized by the anti-Id antibody. In some aspects, such proliferation or other functional response or readout is induced in such cells to a degree that is similar to or greater than that induced by incubation of the cells with an agent and/or conditions that stimulates proliferation of T cells, such as anti-CD3/CD28 beads and/or crosslinked anti-CD3. In some aspects, the methods do not involve crosslinking of the anti-idiotypic antibody. In some aspects of any of the embodiments, the anti-idiotypic agents are capable of inducing the specified proliferation or functional outcome or degree thereof, without crosslinking of the anti-idiotypic antibody. In some aspects, anti-idiotypic agents herein are advantageous in their ability to stimulate or cause a particular functional outcome of T cells or other immune cells expressing the target receptor, without the need to crosslink the anti-Id antibody or use a secondary agent. In some aspects, the

result is achieved with soluble or plate-bound form of the anti-idiotypic antibody. In some aspects, the result is achieved with the anti-idiotypic antibody coupled to a bead.

[0055] In some embodiments, provided is a method of producing a cell composition, including (a) introducing into cells a nucleic acid molecule encoding a chimeric antigen receptor (CAR), thereby generating an input composition; and (b) incubating the input composition with an anti-idiotypic antibody or antigen-binding fragment thereof specific for the antigen receptor of the CAR, thereby producing the cell composition.

[0056] In some aspects, the CAR contains a target antibody that specifically binds to CD19. In some embodiments, the target antibody is the antibody SJ25C1 or an antigen-binding fragment thereof. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof. In some cases, the target antibody is the antibody FMC63 or an antigen-binding fragment thereof. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof specifically binds to a target antibody that is antibody FMC63 of any one of embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof.

[0057] In some of any such embodiments, the introducing in (a) includes introducing the nucleic acid molecule into the cells by viral transduction, transposition, electroporation, or chemical transfection. In some instances, the introducing in (a) includes introducing the nucleic acid molecule in the cells by transduction with a retroviral vector containing the nucleic acid molecule, optionally wherein the viral vector is a retroviral vector or a lentiviral vector. In some aspects, the introducing in (a) includes introducing the nucleic acid molecule in the cells by transposition with a transposon containing the nucleic acid molecule. In some instances, the introducing in (a) includes introducing the nucleic acid molecule in the cells by electroporation or transfection of a vector containing the nucleic acid molecule.

[0058] In some of any such embodiments, the method further includes a step of activating the cells prior to step (a). In some aspects, the step of activating the cells includes contacting the cells with an agonist of CD3 and optionally an agonist of CD28. In some instances, the step of

activating the cells includes contacting the cells with a reagent containing agonistic anti-CD3 and anti-CD28 antibodies.

[0059] In some of any such embodiments, the incubation is performed under conditions in which the anti-idiotypic antibody or antigen-binding fragment thereof binds to the CAR, thereby inducing or modulating a signal in one or more cells in the input composition. In any of such embodiments, the cells contain T cells. In some instances, the T cells contain CD4+ and/or CD8+ T cells.

[0060] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a solid support, which optionally contains or is conjugated to a reagent containing a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof. In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a soluble reagent, which optionally is or contains a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof. In some aspects, the reagent contains a streptavidin mutein.

[0061] In some of any such embodiments, the incubation is for at least or about at least 5 minutes, 10 minutes, 30 minutes, 60 minutes, 2 hours, 6 hours, 12 hours, 24 hours, 36, 48 hours, 72 hours or 96 hours.

[0062] In some of any such embodiments, the input composition contains less than or less than about 60%, less than or less than about 50%, less than or less than about 40%, less than or less than about 30%, less than or less than about 20% or less than or less than about 10% CAR-expressing cells as a percentage of the total cells in the composition.

[0063] In some of any such embodiments, the number of CAR-expressing cells in the output composition is increased by greater than 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold or more compared to the number of CAR-expressing cells in the input composition; and/or the percentage of CAR-expressing in the output composition compared to the total cells in the composition is increased by greater than 10 %, 20 %, 40 %, 50 %, 60 %, 70 %, 80 % or more.

[0064] In some of any such embodiments, prior to the introducing and/or incubating the cells are not selected or enriched for CAR-expressing cells.

[0065] In some of any such embodiments, the target antibody or antigen-binding fragment contains a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some of any such embodiments, the target antibody or antigen-binding fragment contains a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0066] In some embodiments, provided is a method of purifying an antibody or antigen-binding fragment thereof, including (a) contacting a composition containing a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof according to any one of the embodiments described herein or conjugate according to any one of the embodiments described that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and (b) isolating complexes containing the anti-idiotypic antibody. In some embodiments, the method includes (a) contacting a composition containing a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of the embodiments described or the conjugate of any one of the embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and (b) isolating complexes comprising the anti-idiotypic antibody.

[0067] In some embodiments, the complexes containing the anti-idiotypic antibody are isolated by affinity-based separation. In some aspects, the affinity-based separation is immunoaffinity-based separation. In some instances, the affinity-based separation is magnetic-based separation. In some embodiments, the affinity-based separation includes affinity chromatography.

[0068] In some embodiments, provided is a method of identifying an anti-idiotypic antibody or antigen-binding fragment including (a) introducing into a subject a soluble immunization reagent containing an antigen-binding fragment of the target antibody fused to a solubilizing moiety; and (b) identifying an antibody from the subject that specifically binds to the target antibody or the antigen-binding fragment thereof.

[0069] In some of any such embodiments, the antigen-binding fragment contains the variable heavy chain region and/or variable light chain region of the target antibody. In some embodiments, the antigen-binding fragment is a single chain fragment. In some aspects, the antigen-binding fragment is an scFv. In some of any such embodiments, the antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR).

[0070] In some of any such embodiments, the solubilizing moiety is an Fc domain or fragment thereof, which optionally is a human IgG1 Fc. In some aspects, the solubilizing moiety is an Fc domain lacking the hinge region. In some instances, the solubilizing moiety contains the amino acid sequence set forth in SEQ ID NO: 32.

[0071] In some of any such embodiments, identifying the antibody includes (i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas; (ii) screening the hybridomas for production of antibodies that specifically bind the target antibody or the antigen-binding fragment thereof or a chimeric antigen receptor containing the antigen-binding fragment; and (iii) sequencing an antibody from a hybridoma producing an antibody that specifically binds, thereby identifying the anti-idiotypic antibody.

[0072] In some of any such embodiments, the target antibody binds to CD19. In some embodiments, the antigen-binding fragment of the target antibody is derived from antibody SJ25C1, optionally wherein the antigen-binding fragment of the target antibody contains a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some of any such embodiments, the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody SJ25C1, optionally wherein the scFv contains the sequence of amino acids set forth in SEQ ID NO: 28. In some embodiments, the antigen-binding fragment of the target antibody is derived from antibody FMC63, optionally wherein the antigen-binding fragment of the target antibody contains a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31. In some embodiments, the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody FMC63, optionally wherein the scFv contains the sequence of amino acids set forth in SEQ ID NO: 34.

[0073] In some embodiments, there is provided a method of depleting cells, comprising administering, to a subject, a composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of according to any one of the embodiments described herein or conjugate according to any one of the embodiments described that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof. In some embodiments, the method includes administering, to a subject, a composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of the embodiments described herein or conjugate of any one of the embodiments described that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof. In some embodiments, the depletion occurs via antibody-dependent cell-mediated cytotoxicity (ADCC).

[0074] Provided herein is a method of determining the presence or absence of a molecule that binds to a chimeric antigen receptor (CAR), the method including (a) contacting a binding reagent with a sample from a subject having been administered a cell therapy comprising cells engineered with a CAR containing a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof under conditions to form a complex containing the binding reagent and a molecule from the sample that binds to the binding reagent, wherein the binding reagent comprise the extracellular domain of the CAR or a portion thereof containing the target antibody or the antigen-binding fragment thereof; and (b) detecting the presence or absence of the complex, thereby determining the presence or absence of a molecule that binds the CAR. In some embodiments, the method further includes carrying out steps (a) and (b) on a positive control sample and, optionally, determining the presence or absence of the molecule by comparison to the positive control, wherein the positive control sample contains any of the anti-idiotypic antibody or antigen-binding fragment thereof described herein or any of the conjugates described herein that specifically binds to the target antibody or an antigen-binding fragment thereof.

[0075] Provided herein is a method of determining the presence or absence of a molecule that binds to a chimeric antigen receptor (CAR), the method including (a) contacting a binding reagent with a sample from a subject having been administered a cell therapy containing cells engineered with a CAR containing a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof under conditions to form a complex comprising the binding reagent and a molecule from the sample that binds to the binding reagent, wherein the binding reagent contains the extracellular domain of the CAR or a portion of the extracellular domain comprising the target antibody or the antigen-binding fragment thereof; and (b) detecting the presence or absence of the complex. In some embodiments, the method further includes carrying out steps (a) and (b) on a positive control sample and, optionally, determining the presence or absence of the molecule by comparison to the positive control, wherein the positive control sample contains any of the anti-idiotypic antibody or antigen-binding fragment thereof as described herein or any of the conjugates described herein that specifically binds to the target antibody or an antigen-binding fragment thereof.

[0076] In some of any such embodiments, the molecule that binds to the binding reagent is or contains an antibody. In some embodiments, the binding reagent is detectably labeled or is capable of producing a detectable signal. In some instances, the binding reagent is bound to a solid support or is soluble.

[0077] In some of any such embodiments, the complex is detected by an immunoassay. In some example, the immunoassay is an enzyme-linked immunosorbent assay (ELISA), chemiluminescent assay, electrochemiluminescent assay, surface plasmon resonance (SPR)-based biosensor (e.g., BIAcore), flow cytometry, or Western blot. In some embodiments, the immunoassay comprises meso scale discovery. In some cases, the immunoassay is a sandwich assay or bridge assay.

[0078] In some of any such embodiments, the binding reagent is a first binding reagent and detecting the presence or absence of the complex includes: (i) contacting the complex formed in step (a) with a second binding reagent, wherein the second binding reagent (1) contains the extracellular domain of the CAR or a portion thereof comprising the target antibody or the antigen-binding fragment thereof, and (2) is detectably labeled or is capable of producing a detectable signal; and (ii) assessing the presence or absence of the detectable signal. In some

aspects, the first binding reagent is bound to a solid support, optionally wherein first binding reagent is linked, directly or indirectly, to a biotin and/or bound to a solid support through a streptavidin; and/or the second binding reagent is soluble. In some cases, the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

[0079] In some of any such embodiments, the detectable label is or contains a fluorescent label, a chemiluminescent label, an electroluminescent label, a colorimetric label, a bioluminescent label or a radiolabel; and/or the detectable signal is or contains a fluorescent signal, chemiluminescent signal, electroluminescent signal, colorimetric signal, a bioluminescent signal or a radioactive signal. In some of any such embodiments, the detectable label is or contains a SULFO-TAG.

[0080] In some of any such embodiments, the antigen-binding fragment of the target antibody contains the variable heavy chain region and/or variable light chain region of the target antibody. In some of any such embodiments, the antigen-binding fragment of the target antibody is a single chain fragment. In some embodiments, the antigen-binding fragment of the target antibody is an scFv.

[0081] In some of any such embodiments, the sample comprises whole blood, serum or plasma.

[0082] Provided herein is an article of manufacture containing any of the anti-idiotypic antibodies or antigen-binding fragment thereof described herein or any of the conjugates described, and instructions for using the anti-idiotypic antibody to detect an SJ25C1 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof; to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) containing the antibody SJ25C1 or an antigen-binding fragment thereof; to stimulate an input composition comprising cells expressing a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof.

[0083] Provided herein is an article of manufacture containing any of the anti-idiotypic antibodies or antigen-binding fragment thereof as described herein or any of the conjugates described herein, and instructions for using the anti-idiotypic antibody to detect an FMC63 antibody or antigen-binding fragment thereof or a chimeric antigen receptor containing the

FMC63 antibody or antigen-binding fragment thereof; to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody FMC63 or an antigen-binding fragment thereof; to stimulate an input composition comprising cells expressing a chimeric antigen receptor containing the FMC63 antibody or antigen-binding fragment thereof.

[0084] Provided herein is an article of manufacture containing a binding reagent including the extracellular domain of a chimeric antigen receptor (CAR) containing a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, said extracellular domain or portion thereof containing the target antibody or antigen-binding fragment thereof; and an anti-idiotypic antibody or antigen-binding fragment described herein or the any of the conjugates described herein. In some embodiments, the binding reagent is a first binding reagent and the article of manufacture further includes a second binding reagent containing the extracellular domain or portion thereof of the CAR.

[0085] In some of any such embodiments, the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

[0086] In some of any such embodiments, the article of manufacture further includes instructions for using the binding reagent, optionally the first and second binding reagent, for assaying a sample for the presence or absence of a molecule that binds to the binding reagent using an immunoassay, optionally wherein the immunoassay is a bridge or sandwich immunoassay, optionally wherein the sample is from a subject having been administered a cell therapy including cells engineered with a CAR containing a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof.

[0087] Provided herein is an article of manufacture including a binding reagent containing the extracellular domain of a chimeric antigen receptor (CAR) containing a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, said extracellular domain or portion thereof containing the target antibody or antigen-binding fragment thereof; and an anti-idiotypic antibody or antigen-binding fragment described herein or a conjugate described herein.

[0088] In some embodiments, the binding reagent is a first binding reagent and the article of manufacture further contains a second binding reagent containing the extracellular domain or portion thereof of the CAR. In some aspects, the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

[0089] In some of any such embodiments, the article of manufacture further contains instructions for using the binding reagent, optionally the first and second binding reagent, for assaying a sample for the presence or absence of a molecule that binds to the binding reagent using an immunoassay, optionally wherein the immunoassay is a bridge or sandwich immunoassay, optionally wherein the sample is from a subject having been administered a cell therapy including cells engineered with a CAR comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof.

[0090] In some embodiments, the binding reagent, optionally the first and/or second binding reagent, is detectably labeled or capable of producing a detectable signal. In some cases, one of the first and second binding reagent is attached to a solid support or is capable of being attached to a solid support and the other of the first and second binding reagent is detectable label or is capable of producing a detectable signal. In some embodiments, the article of manufacture further includes a solid support, optionally wherein the one of the first and second binding reagent is linked, directly or indirectly to biotin, and the solid support comprises a streptavidin-coated surface.

Brief Description of the Drawings

[0091] **FIG. 1** shows the results of flow cytometry to assess the functional activity of an SJ25C1-derived scFv-specific anti-idiotypic antibody clone A-1 (anti-ID A-1) to stimulate Erk1/2 phosphorylation in Jurkat cells engineered with an SJ25C1-derived CAR. Activation with an anti-CD3 antibody was included as a positive control. Unstimulated or isotype control stimulated cells were included as negative controls.

[0092] **FIG. 2A-B** shows results from an assay for the proliferation of T cell expressing a CAR containing either an SJ25C1-derived binding domain (FIG. 2A) or an FMC63-derived binding domain (FIG. 2B), as assessed by dye dilution using flow cytometry following

stimulation with an anti-CD3 antibody (OKT3), anti-ID A-1, or anti-ID B-1 anti-idiotypic antibody. Unstimulated cells were included as a negative control.

[0093] FIG. 2C shows results from an assay for the mock transduced and CAR transduced proliferation of T cells (labeled with dye), following culture in the presence of stimulated by plate-bound anti-idiotypic antibody recognizing the binding domain of the CAR as assessed by dye dilution using flow cytometry.

[0094] FIG. 3 shows results for an assessment of the expression of two markers of T cell activation, CD69 and CD25, in CD4⁺ or CD8⁺ T cells expressing a CAR with variable regions derived from SJ25C1 as assessed by flow cytometry following stimulation with plate-bound anti-CD3 antibody (OKT3), anti-ID A-1 or anti-ID B-1.

[0095] FIG. 4 shows results for an assessment of the expression of two markers of T cell activation, CD69 and CD25, in CD4⁺ or CD8⁺ T cells expressing a CAR with a binding domain having variable regions derived from FMC63, as assessed by flow cytometry following stimulation with plate-bound anti-CD3 antibody (OKT3), anti-ID A-1 or anti-ID B-1, or a negative control non-target anti-idiotypic antibody. Unstimulated cells were included as a negative control.

[0096] FIG. 5 shows results from a bridge ELISA for detecting anti-CAR antibodies using anti-ID B-1 and anti-ID B-2 antibodies recognizing the CAR binding domain, at a range of concentrations, as positive controls.

[0097] FIG. 6 and FIG. 7 shows fold expansion and cumulative cell numbers of EGFRt⁺/CD4⁺ T cells or EGFRt⁺/CD8⁺ T cells, respectively, stimulated with the indicated ratio of beads coated with anti-idiotypic antibody (anti-ID B-1) or control CD3/CD28 antibody coated beads in the presence or absence of cytokines.

[0098] FIG. 8 shows PD-1 expression levels of CD4⁺ T cells positive for staining with the anti-EGFR antibody, and thus positive for the transduction marker EGFRt, after stimulation with the indicated ratio of beads coated with anti-idiotypic antibody (anti-ID B-1) or control CD3/CD28 antibody coated beads in the presence or absence of cytokines as assessed by flow cytometry at days 3, 7, 10 and 14 of culture.

[0099] **FIG. 9** shows the viability of CD4⁺ or CD8⁺ T cells expressing an FMC63-derived CAR as assessed by flow cytometry following stimulation with the indicated ratio of beads coated with anti-idiotypic antibody (anti-ID B-1) or control CD3/CD28 antibody coated beads in the presence or absence of cytokines as assessed by flow cytometry at days 3, 7, 10 and 14 of culture.

[0100] **FIG. 10A** shows intracellular cytokine staining for IL-2, TNF α , and IFN γ of T cells expressing an FMC63-derived CAR following stimulation with FMC63-derived scFv-specific anti-idiotypic antibody (anti-ID B-1) coated beads. Shown are results for CD8⁺ T cells positive or negative for the EGFRt surrogate transduction marker (EGFR⁺ or EGFRt⁻).

[0101] **FIG. 10B** shows intracellular cytokine staining for IL-2, TNF α , and IFN γ of T cells expressing an FMC63-derived CAR following stimulation with antigen-expressing K562-CD19 cells. Shown are results of CD8⁺ T cells positive for the anti-EGFR antibody as a surrogate for CAR expression.

[0102] **FIG. 11** shows the number of population doublings in a serial stimulation assay over a 14 day culture period of T cells expressing an FMC63-derived scFv-derived CAR following stimulation with the indicated ratio of beads coated with anti-idiotypic antibody (anti-ID B-1) or control anti-CD3/anti-CD28 antibody coated beads in the presence or absence of cytokines. Shown are results for CD4⁺ T cells positive for the EGFRt surrogate transduction marker (EGFRt⁺/CD4⁺) or CD8⁺ T cells positive for EGFRt (EGFRt⁺/CD8⁺).

[0103] **FIG. 12A-12C** show results following stimulation of CD4⁺ or CD8⁺ T cells expressing an FMC63-derived CAR, cultured alone or as a co-culture, with FMC63-derived scFv-specific anti-idiotypic antibody (anti-ID B-1) coated beads. Results are shown for two different donors. **FIG. 12A** depicts the fold-expansion of CD4⁺ T cells or CD8⁺ T cells in the cultures that were positive for the EGFRt surrogate transduction marker (EGFRt⁺/CD4⁺ or EGFRt⁺/CD8⁺). **FIG. 12B** shows the frequency of CD4⁺ T cells or CD8⁺ T cells in the cultures that were positive for EGFRt (EGFRt⁺/CD4⁺ or EGFRt⁺/CD8⁺). **FIG. 12C** shows the viability of CD4⁺ T cells or CD8⁺ T cells in the cultures.

[0104] **FIG. 13A** and **13B** show flow cytometry results for T cell surface markers at days 5, 7 and 9 of culture following stimulation of CD4⁺ or CD8⁺ T cells expressing an FMC63-derived CAR, cultured alone or as a co-culture, with FMC63-derived scFv-specific anti-idiotypic

antibody (anti-ID B-1) coated beads. **FIG. 13A** shows surface expression of PD-1 on CD4+ T cells or CD8+ T cells in the cultures that were positive for the EGFRt surrogate transduction marker (EGFRt+/CD4+ or EGFRt+/CD8+). **FIG. 13B** shows surface expression of CD25 on CD4+ T cells or CD8+ T cells in the cultures that were positive for the anti-EGFR antibody as a surrogate for CAR expression (EGFRt+/CD4+ or EGFRt+/CD8+).

[0105] **FIG. 14A** shows intracellular cytokine levels of TNF α , IFN γ , and IL-2 as assessed by flow cytometry of CD4+ or CD8+ T cells present in a thawed composition containing T cells expressing an FMC63-derived CAR that had been expanded in culture either with CD19 expressing K562 cells or with PMA/Ionomycin. Shown are the level of the cytokines in CD4+ and CD8+ T cells, alone or as a co-culture, at thaw (d=0) or after a further culture for an additional 9 days in the presence of anti-ID B-1 conjugated beads.

[0106] **FIG. 14B** shows the frequency of cells positive for CD25 or Ki67 as assessed by flow cytometry of CD4+ or CD8+ T cells present in a thawed composition containing T cells expressing an FMC63-derived CAR that had been expanded in culture either with CD19 expressing K562 cells or with PMA/Ionomycin. Shown are the level of the markers in CD4+ and CD8+ T cells, alone or as a co-culture, at thaw (d=0) or after a further culture for an additional 9 days in the presence of anti-ID B-1 conjugated beads.

[0107] **FIG. 15A** and **15B** show a graph depicting results of staining cells with anti-EGFR antibody or FMC63-derived scFv-specific anti-idiotypic antibodies (anti-ID B-1 and anti-ID B-2). **FIG. 15A** shows a graph depicting mean fluorescent intensity of cells that were stained with different concentrations of the antibodies. Cells included a mixture of PBMCs and CAR expressing cells. **FIG. 15B** shows a graph depicting the percentage of CAR expressing cells in de FMC63-derived scFv-specific anti-idiotypic antibody (anti-ID B-1) detected in cells that were stained with different concentrations of antibodies. The cells included a mixture of PBMCs and CAR expressing cells and PBMCs alone.

Detailed Description

[0108] Provided herein are agents such as anti-idiotypic antibodies and antigen-binding fragments (such as single chain fragments, including scFvs) that specifically recognize anti-CD19 antibody moieties (such as anti-CD19 antibody moieties present in recombinant receptors,

including chimeric antigen receptors). Also provided are uses and methods of use thereof, and compositions and articles of manufacture including such agents, including for specifically identifying, selecting, and/or stimulating and/or activating cells expressing or including the target antibodies or fragments such as anti-CD19 CAR T cells. In some embodiments, the provided antibodies can be used for specific identification and/or selection of various anti-CD19 CARs, such as CARs bound to or expressed on a cell surface, and can also be used to specifically activate cells expressing target CARs, such as CAR T cells. In some embodiments, provided are antibodies that are specific to the anti-CD19 antibody designated SJ25C1 or FMC63, or an antibody fragment derived therefrom, including antibodies and CARs containing variable regions derived from such antibodies, and/or an antibody containing an idiotope contained therein.

[0109] In some aspects, the provided anti-idiotypic antibodies offer advantages compared to conventional reagents for detecting, identifying, manipulating and/or affecting and/or engineering cells that express a CAR, and in particular a CAR containing an anti-CD19 antibody scFv extracellular domain or one containing the recognized idiotype. In certain available methods detection of the presence or absence or amount of CAR or CAR-expressing cells (and/or stimulation or manipulation of the CAR), in a sample, is carried out by assessing the presence or absence or amount of a surrogate molecule, such as one included in the construct encoding the CAR and thus serving as an indirect or surrogate marker for its expression. In certain available methods, detection is carried out using a generic antibody reagent and/or a reagent that is not specific for the particular CAR assessed, e.g., as compared to other CARs that may have similar or identical domains other than the antigen-binding region; for example, such antibodies may include anti-species antibodies recognizing spacer or other domains from the species from which a CAR domain was derived, and/or antibodies recognizing particular components used in spacer regions of the target and also other chimeric receptors. In certain available methods designed to detect the presence or absence of CARs, detection is carried out using an agent recognizing a CAR constant region. In certain available methods, CAR cells are stimulated through the use of general reagents, such as anti-CD3/CD28 recognizing agents. Certain methods use a recombinant ligand of the CAR (e.g., CD19-Fc). Such methods in certain contexts may not be entirely satisfactory and/or have certain limitations. In some cases, CAR

ligands, such as CD19, may not always be entirely effective, e.g., for use in complex flow cytometry panels. Improved methods and agents are needed, including those providing improved sensitivity and/or selectivity. Provided herein are embodiments meeting such needs.

[0110] The provided anti-idiotypic antibodies and antigen-binding fragments in some embodiments overcome challenges of low binding affinity associated with target antibody ligands and non-specific binding associated with antibody reagents directed to target antibody constant regions, providing a reagent with both high affinity and specificity for its target antibody or antigen binding fragment thereof. In some embodiments, the provided antibodies exhibit greater specificity and binding affinity for their target antibodies or antigen-binding fragments, such as the anti-CD19 antibody designated SJ25C1 or FMC63, compared to CD19-Fc and other reagents currently available for detecting or identifying the CAR.

[0111] Furthermore, in certain embodiments, anti-idiotypic antibodies and antigen-binding fragments that may be selected as agonists or antagonists of chimeric receptors comprising their target antibodies or antigen-binding fragments, allowing for selective detection, isolation, ablation and/or depletion (for example, killing via antibody-dependent cell-mediated cytotoxicity, ADCC), and/or stimulation or activation of cells with such chimeric receptors bound to or expressed on their surface. Provided herein are anti-idiotypic antibody agonists that exhibit activity to stimulate, such as activate, a CAR containing an extracellular binding domain derived from anti-CD19 antibody designated SJ25C1 or FMC63. In some aspects, such antibodies can be used in methods of stimulating and expanding specific CAR-expressing cells, including in processes for generating and preparing the CAR-expressing cells.

[0112] Also provided herein are nucleic acids encoding the provided anti-idiotypic antibodies and fragments, and cells, such as recombinant cells, expressing and for production of these anti-idiotypic antibodies and fragments. Also provided are methods of making and using the anti-idiotypic antibodies and fragments, as well as cells expressing or containing the anti-idiotypic antibodies and fragments.

[0113] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in

the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0114] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. ANTI-IDIOTYPE ANTIBODIES

[0115] Provided in some aspects are binding molecules, such as anti-idiotypic antibodies or antigen-binding fragments (“anti-IDs”) that specifically recognize a target anti-CD19 antibody moiety. In some embodiments, the provided antibodies recognize a target anti-CD19 antibody that is SJ25C1 or an antigen-binding fragment thereof or is an antibody or antigen-binding fragment derived from SJ25C1. In some embodiments, the provided antibodies recognize a target anti-CD19 antibody that is FMC63 or an antigen-binding fragment thereof is an antibody or antigen-binding fragment derived from FMC63.

[0116] SJ25C1 is a mouse monoclonal IgG1 antibody raised against Nalm-1 and -16 cells expressing CD19 of human origin (Ling, N. R., *et al.* (1987). *Leucocyte typing III*. 302). The SJ25C1 antibody comprises CDRH1, H2 and H3 set forth in SEQ ID NOS: 114-116, respectively, and CDRL1, L2 and L3 sequences set forth in SEQ ID NOS: 117-119, respectively. The SJ25C1 antibody comprises the heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO: 23 and the light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 24.

[0117] In some embodiments, the target antibody is SJ25C1 or an antibody-derived from SJ25C1. In some embodiments, the antibody derived from SJ25C1 is an antibody or antigen-binding fragment that comprises the V_H and/or V_L of SJ25C1, the idiotype of SJ25C1, the paratope of SJ25C1, or one or more complementarity determining regions (CDRs) of SJ25C1. In some embodiments, the target antibody that is SJ25C1 or an antibody-derived from SJ25C1 is an antibody or antigen-binding fragment comprising the V_H of SJ25C1 set forth in SEQ ID NO:23, or a variant thereof having at least 90% sequence identity to SEQ ID NO:23, and/or the V_L of SJ25C1 set forth in SEQ ID NO:24, or a variant thereof having at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the target antibody that is SJ25C1 or an antibody-derived from SJ25C1 is an antibody or antigen-binding fragment comprising the V_H of SJ25C1

set forth in SEQ ID NO:23, or a variant thereof having at least 90% sequence identity to SEQ ID NO:23, and the V_L of SJ25C1 set forth in SEQ ID NO:24, or a variant thereof having at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the antibody or antigen-binding fragment comprises the V_H and V_L of SJ25C1 set forth in SEQ ID NO:23 and SEQ ID NO:24, respectively. In some embodiments, the variant has at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23 and/or SEQ ID NO:24.

[0118] In some embodiments, the target antibody that is SJ25C1 or an antibody-derived from SJ25C1 is an antibody or antigen-binding fragment comprising one or more heavy chain CDRs (CDR-H) of SJ25C1 V_H set forth in SEQ ID NO:23, such as set forth in SEQ ID NOS: 114-116 and/or one or more light chain CDRs (CDR-Ls) of SJ25C1 V_L set forth in SEQ ID NO:24, such as set forth in SEQ ID NOS: 117-119. In some embodiments, the antibody or antigen-binding fragment comprises CDR-H3 of SJ25C1 (e.g. set forth in SEQ ID NO: 116) and/or CDR-L3 of SJ25C1 (e.g. set forth in SEQ ID NO: 119). In some embodiments, the antibody or antigen-binding fragment comprises CDR-H3 and CDR-L3 of SJ25C1 (e.g. set forth in SEQ ID NO:116 and 119, respectively). In some embodiments, the target antibody that is SJ25C1 or an antibody-derived from SJ25C1 is an antibody or antigen-binding fragment comprises one or more of CDR-H1, CDR-H2, and CDR-H3 of SJ25C1 (e.g. set forth in SEQ ID NOS: 114, 115, 116, respectively) and/or one or more of CDR-L1, CDR-L2, and CDR-L3 of SJ25C1 (e.g. set forth in SEQ ID NOS: 117, 118, 119, respectively). In some embodiments, the target antibody that is SJ25C1 or an antibody-derived from SJ25C1 is an antibody or antigen-binding fragment comprises CDR-H1, CDR-H2, and CDR-H3 of SJ25C1 (e.g. set forth in SEQ ID NOS: 114, 115, 116, respectively) and/or CDR-L1, CDR-L2, and CDR-L3 of SJ25C1 (e.g. set forth in SEQ ID NOS: 117, 118, 119, respectively). In some embodiments, the target antibody that is SJ25C1 or an antibody-derived from SJ25C1 is an antibody or antigen-binding fragment comprises CDR-H1, CDR-H2, and CDR-H3 of SJ25C1 (e.g. set forth in SEQ ID NOS: 114, 115, 116, respectively) and CDR-L1, CDR-L2, and CDR-L3 of SJ25C1 (e.g. set forth in SEQ ID NOS: 117, 118, 119, respectively). In some embodiments, the antibody or antigen-binding fragment comprises an antigen-binding fragment, such as a fragment antigen-binding (Fab), a F(ab')₂, a Fab', a fragment variable (Fv), or a single chain Fv (scFv). *See for example* Bejcek, B. E., *et al.* (1995). *Cancer research*. 55(11): 2346-2351.

[0119] FMC63 is a mouse monoclonal IgG1 antibody raised against JVM3 cells expressing CD19 of human origin (Nicholson *et al.* (1997). *Molecular Immunology*. 34(16-17):1157-1165). The FMC63 antibody comprises CDRH1, H2 and H3 set forth in SEQ ID NOS: 120-122, respectively, and CDRL1, L2 and L3 sequences set forth in SEQ ID NOS: 123-125, respectively. The FMC63 antibody comprises the heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO: 30 and the light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 31.

[0120] In some embodiments, the target antibody is FMC63 or an antibody-derived from FMC63. In some embodiments, the antibody derived from FMC63, is an antibody or antigen-binding fragment that comprises the V_H and/or V_L of FMC63, the idiotype of FMC63, the paratope of FMC63, or one or more complementarity determining regions (CDRs) of FMC63. In some embodiments, the target antibody that is FMC63 or an antibody-derived from FMC63 is an antibody or antigen-binding fragment comprising the V_H of FMC63 set forth in SEQ ID NO: 30, or a variant thereof having at least 90% sequence identity to SEQ ID NO:30, and/or the V_L of FMC63 set forth in SEQ ID NO:31, or a variant thereof having at least 90% sequence identity to SEQ ID NO:31. In some embodiments, the target antibody that is FMC63 or an antibody-derived from FMC63 is an antibody or antigen-binding fragment comprises the V_H of FMC63 set forth in SEQ ID NO:30, or a variant thereof having at least 90% sequence identity to SEQ ID NO:30, and the V_L of FMC63 set forth in SEQ ID NO:31, or a variant thereof having at least 90% sequence identity to SEQ ID NO:31. In some embodiments, the antibody or antigen-binding fragment comprises the V_H and V_L of FMC63 set forth in SEQ ID NO:30 and SEQ ID NO:31, respectively. In some embodiments, the variant has at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:30 and/or SEQ ID NO:31.

[0121] In some embodiments, the target antibody that is FMC63 or an antibody-derived from FMC63 is an antibody or antigen-binding fragment comprises one or more heavy chain CDRs (CDR-H) of FMC63 V_H set forth in SEQ ID NO:30, such as set forth in SEQ ID NOS: 120-122 and/or one or more light chain CDRs (CDR-Ls) of FMC63 V_L set forth in SEQ ID NO:31, such as set forth in SEQ ID NOS: 123-125. In some embodiments, the antibody or antigen-binding fragment comprises CDR-H3 of FMC63 (e.g. set forth in SEQ ID NO: 122) and/or CDR-L3 of FMC63 (e.g. set forth in SEQ ID NO: 125). In some embodiments, the

antibody or antigen-binding fragment comprises CDR-H3 (e.g. set forth in SEQ ID NO: 122) and CDR-L3 of FMC63 (e.g. set forth in SEQ ID NO: 125). In some embodiments, the antibody or antigen-binding fragment comprises one or more of CDR-H1, CDR-H2, and CDR-H3 of FMC63 (set forth in SEQ ID NOs: 120, 121, 122, respectively) and/or one or more of CDR-L1, CDR-L2, and CDR-L3 of FMC63 (set forth in SEQ ID NOs: 123, 124, 125, respectively). In some embodiments, the antibody or antigen-binding fragment comprises CDR-H1, CDR-H2, and CDR-H3 of FMC63 (set forth in SEQ ID NOs: 120, 121, 122, respectively) and/or CDR-L1, CDR-L2, and CDR-L3 of FMC63 (set forth in SEQ ID NOs: 123, 124, 125, respectively). In some embodiments, the antibody or antigen-binding fragment comprises CDR-H1, CDR-H2, and CDR-H3 of FMC63 (set forth in SEQ ID NOs: 120, 121, 122, respectively) and CDR-L1, CDR-L2, and CDR-L3 of FMC63 (set forth in SEQ ID NOs: 123, 124, 125, respectively). In some embodiments, the antibody or antigen-binding fragment comprises an antigen-binding fragment, such as a fragment antigen-binding (Fab), a F(ab')₂, a Fab', a fragment variable (Fv), or a single chain Fv (scFv).

[0122] In some embodiments, the provided anti-idiotypic antibodies include antibodies that specifically bind to a variable domain (Fv), such as a single chain Fv (scFv), derived from SJ25C1 or FMC63. In some embodiments, the anti-idiotypic antibodies specifically bind to a particular epitope or region of an Fv, generally an epitope or region comprising one or more complementarity determining regions. In some embodiments, the anti-idiotypic antibodies specifically bind to an epitope or region overlapping an Fv paratope.

[0123] In some embodiments, the provided anti-idiotypic antibodies include those that specifically bind to an anti-CD19 moiety derived from SJ25C1 or FMC63 that is contained as part of the extracellular domain of a target chimeric antigen receptor (CAR). In some embodiments, the target CAR contains an antigen-binding portion that contains the SJ25C1 or FMC63 antibody molecule or antigen-binding fragment or portion of the SJ25C1 or FMC63 antibody. In some embodiments, the target CAR includes an antigen-binding domain that is an scFv derived from the VH and VL chains of the antibody SJ25C1 or FMC63. In some embodiments, there is provided an anti-idiotypic antibody that specifically binds to an anti-CD19 CAR that contains an scFv derived from antibody SJ25C1 or FMC63. Exemplary features of CARs are described further below.

[0124] The term “antibody” herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, e.g., bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term “antibody” should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0125] The term “anti-idiotypic antibody” refers to an antibody, including antigen-binding fragments thereof, that specifically recognizes, is specifically targeted to, and/or specifically binds to an idiotope of an antibody, such as an antigen-binding fragment. The idiotopes of an antibody may include, but are not necessarily limited to, residues within one or more of complementarity determining region(s) (CDRs) of the antibody, variable regions of the antibody, and/or partial portions or portions of such variable regions and/or of such CDRs, and/or any combination of the foregoing. The CDR may be one or more selected from the group consisting of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3. The variable regions of the antibody may be heavy chain variable regions, light chain variable regions, or a combination of the heavy chain variable regions and the light chain variable regions. The partial fragments or portions of the heavy chain variable regions and/or the light chain variable regions of the antibody may be fragments including 2 or more, 5 or more, or 10 or more contiguous amino acids, for example, from about 2 to about 100, from about 5 to about 100, from about 10 to about 100, from about 2 to about 50, from about 5 to about 50, or from about 10 to about 50 contiguous amino acids within the heavy chain variable regions or the light chain variable regions of the antibody; the idiotope may include multiple non-contiguous stretches of amino acids. The partial fragments of the heavy chain variable regions and the light chain variable

regions of the antibody may be fragments including 2 or more, 5 or more, or 10 or more contiguous amino acids, for example, from about 2 to about 100, from about 5 to about 100, from about 10 to about 100, from about 2 to about 50, from about 5 to about 50, or from about 10 to about 50 contiguous amino acids within the variable regions, and in some embodiments contain one or more CDRs or CDR fragments. The CDR fragments may be consecutive or non-consecutive 2 or more, or 5 or more amino acids within the CDR. Therefore, the idiotopes of the antibody may be from about 2 to about 100, from about 5 to about 100, from about 10 to about 100, from about 2 to about 50, from about 5 to about 50, or from about 10 to about 50 contiguous amino acids containing one or more CDR or one or more CDR fragments within the heavy chain variable regions or the light chain variable regions of the antibody. In another embodiment, the idiotopes may be a single amino acid which is located at the variable regions of the antibody, for example, CDR sites.

[0126] In some embodiments, the idiotope is any single antigenic determinant or epitope within the variable portion of an antibody. In some cases it can overlap the actual antigen-binding site of the antibody, and in some cases it may comprise variable region sequences outside of the antigen-binding site of the antibody. The set of individual idiotopes of an antibody is in some embodiments referred to as the “idiotype” of such antibody.

[0127] The terms “complementarity determining region,” and “CDR,” synonymous with “hypervariable region” or “HVR,” are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). “Framework regions” and “FR” are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4).

[0128] The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme), Al-Lazikani et al.,

(1997) JMB 273,927-948 (“Chothia” numbering scheme), MacCallum et al., J. Mol. Biol. 262: 732-745 (1996), “Antibody-antigen interactions: Contact analysis and binding site topography,” J. Mol. Biol. 262, 732-745.” (“Contact” numbering scheme), Lefranc MP et al., “IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains,” Dev Comp Immunol, 2003 Jan;27(1): 55-77 (“IMGT” numbering scheme), and Honegger A and Plückthun A, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool,” J Mol Biol, 2001 Jun 8;309(3): 657-70, (“Aho” numbering scheme).

[0129] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, “30a,” and deletions appearing in some antibodies. The two schemes place certain insertions and deletions (“indels”) at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

[0130] Table 1, below, lists exemplary position boundaries of CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-L1 located between CDR-L1 and CDR-L2, and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

Table 1

CDR	Kabat	Chothia	Contact
CDR-L1	L24--L34	L24--L34	L30--L36
CDR-L2	L50--L56	L50--L56	L46--L55
CDR-L3	L89--L97	L89--L97	L89--L96
CDR-H1 (Kabat Numbering ¹)	H31--H35B	H26--H32..34	H30--H35B
CDR-H1	H31--H35	H26--H32	H30--H35

(Chothia Numbering ²)			
CDR-H2	H50--H65	H52--H56	H47--H58
CDR-H3	H95--H102	H95--H102	H93--H101

1 - Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD

2 - Al-Lazikani et al., (1997) JMB 273,927-948

[0131] Thus, unless otherwise specified, a "CDR" or "complementary determining region," or individual specified CDRs (e.g., "CDR-H1, CDR-H2), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes. For example, where it is stated that a particular CDR (e.g., a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given V_H or V_L amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (e.g., CDR-H3) within the variable region, as defined by any of the aforementioned schemes. In some embodiments, specified CDR sequences are specified.

[0132] Likewise, unless otherwise specified, a FR or individual specified FR(s) (e.g., FR-H1, FR-H2), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a particular CDR, FR, or FRs or CDRs is specified, such as the CDR as defined by the Kabat, Chothia, or Contact method. In other cases, the particular amino acid sequence of a CDR or FR is given.

[0133] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, e.g., Portolano et al., J. Immunol. 150: 880-887 (1993); Clarkson et al., Nature 352: 624-628 (1991).

[0134] Among the provided antibodies are antibody fragments. An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0135] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody.

[0136] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, *e.g.*, peptide linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some aspects, the antibody fragments are scFvs.

[0137] A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all framework regions (FRs) amino acid residues are derived from human FRs. In some embodiments, the humanized forms of a non-human antibody, *e.g.*, a murine antibody, are chimeric antibodies that contain minimal sequences derived from non-human immunoglobulin. In certain embodiments, the humanized antibodies are antibodies from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region (FR) from a human immunoglobulin molecule. In some embodiments, a humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding

residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity. (See, e.g., Queen, U.S. Pat. No. 5,585,089 and Winter, U.S. Pat. No. 5,225,539.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art.

[0138] In certain embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a heavy chain variable region of the recipient are replaced by residues from a heavy chain variable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. In some embodiments, a nucleic acid sequences encoding human variable heavy chains and variable light chains are altered to replace one or more CDR sequences of the human (acceptor) sequence by sequence encoding the respective CDR in the nonhuman antibody sequence(donor sequence). In some embodiments, the human acceptor sequence may comprise FR derived from different genes. In particular embodiments, a humanized antibody will contain substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. In some embodiments, the humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, e.g., Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409, incorporated by reference herein. In some embodiments, provided herein are humanized anti-idiotypic antibodies.

[0139] In particular embodiments, an antibody, e.g., an anti-idiotypic antibody, is humanized. In certain embodiments, the antibody is humanized by any suitable known means. For example, in some embodiments, a humanized antibody can have one or more amino acid residues

introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. In particular embodiments, humanization can be essentially performed by following the method of Winter and co-workers (Jones et al. (1986) Nature 321:522-525; Riechmann et al. (1988) Nature 332:323-327; Verhoeven et al. (1988) Science 239:1534-1536), such as by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In certain embodiments, the humanized antibody is a human antibody in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0140] Sequences encoding full length antibodies can be subsequently obtained by joining the rendered variable heavy and variable light chain sequences to human constant heavy chain and constant light chain regions. Suitable human constant light chain sequences include kappa and lambda constant light chain sequences. Suitable human constant heavy chain sequences include IgG1, IgG2 and sequences encoding IgG1 mutants which have rendered immune-stimulating properties. Such mutants may have a reduced ability to activate complement and/or antibody dependent cellular cytotoxicity and are described in U.S. Pat. No. 5,624,821; WO 99/58572, U.S. Pat. No. 6,737,056. A suitable constant heavy chain also includes an IgG1 comprising the substitutions E233P, L234V, L235A, A327G, A330S, P331S and a deletion of residue 236. In another embodiment, the full length antibody comprises an IgA, IgD, IgE, IgM, IgY or IgW sequence.

[0141] Suitable human donor sequences can be determined by sequence comparison of the peptide sequences encoded by the mouse donor sequences to a group of human sequences, preferably to sequences encoded by human germ line immunoglobulin genes or mature antibody genes. A human sequence with a high sequence homology, preferably with the highest homology determined may serve as the acceptor sequence to for the humanization process.

[0142] In addition to the exchange of human CDRs for mouse CDRs, further manipulations in the human donor sequence may be carried out to obtain a sequence encoding a humanized antibody with optimized properties (such as affinity of the antigen).

[0143] Furthermore the altered human acceptor antibody variable domain sequences may also be rendered to encode one or more amino acids (according to the Kabat numbering system) of position 4, 35, 38, 43, 44, 46, 58, 62, 64, 65, 66, 67, 68, 69, 73, 85, 98 of the light variable region and 2, 4, 36, 39, 43, 45, 69, 70, 74, 75, 76, 78, 92 of the heavy variable region corresponding to the non-human donor sequence (Carter and Presta, U.S. Pat. No. 6,407,213)

[0144] In particular embodiments, it is generally desirable that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, in some embodiments, the humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and imported sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0145] In particular embodiments, choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework for the humanized antibody. See, e.g., Sims et al. (1993) J. Immunol. 151:2296; Chothia et al. (1987) J. Mol. Biol. 196:901. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies. See, e.g., Carter et al. (1992) Proc. Natl. Acad. Sci. USA, 89:4285; Presta et al. (1993) J. Immunol., 151:2623.

[0146] Among the provided antibodies are human antibodies. A “human antibody” is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human.

[0147] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal’s chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

[0148] Among the provided antibodies are monoclonal antibodies, including monoclonal antibody fragments. The term “monoclonal antibody” as used herein refers to an antibody obtained from or within a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical, except for possible variants containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. The term is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be made by a variety of techniques, including but not limited to generation from a hybridoma, recombinant DNA methods, phage-display and other antibody display methods.

A. SJ25C1-derived Antibodies

[0149] In some embodiments, provided are anti-idiotypic antibodies specific to a target anti-CD19 antibody that is or is derived from antibody SJ25C1 or an antigen-binding fragment thereof. In some embodiments, the provided antibodies or antigen-binding fragments are specific to an SJ25C1-derived scFv.

[0150] In some embodiments, provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include a heavy chain variable (V_H) region comprising at least 90% sequence identity to the V_H region amino acid sequence set forth in SEQ ID NO: 1, such as at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

[0151] In some embodiments, provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include a heavy chain variable (V_H) region containing a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence set forth in SEQ ID NO: 11 or 84 and/or a CDR-H3 contained within the heavy chain variable (V_H) sequence set forth in SEQ ID NO: 1.

[0152] In some of any such embodiments, the V_H region includes a heavy chain complementarity determining region 1 (CDR-H1) comprising the amino acid sequence set forth in SEQ ID NO: 9, 78, 79, or 80, and/or a CDR-H1 contained within the V_H sequence set forth in SEQ ID NO: 1; and/or a heavy chain complementarity determining region 2 (CDR-H2) comprising the amino acid sequence set forth in SEQ ID NO: 10, 81, 82, or 83 and/or a CDR-H2 contained within the V_H sequence set forth in SEQ ID NO: 1.

[0153] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include a heavy chain variable (V_H) region comprising a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and CDR-H3, wherein the CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 9, 78, 79, or 80; the CDR-H2 comprises the amino acid sequence set forth in SEQ ID NO: 10, 81, 82, or 83; and/or the CDR-H3 comprises the amino acid sequence set forth in SEQ ID NO: 11 or 84. In some embodiments, provided are antibodies or antigen-binding fragments thereof that include a CDR-H1 having the amino acid sequence set forth in SEQ ID NO: 9, 78, 79, or 80; a CDR-H2 having the amino acid sequence set forth in SEQ ID NO: 10, 81, 82, or 83; and a CDR-H3 having the amino acid sequence set forth in SEQ ID NO: 11 or 84.

[0154] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that includes a heavy chain complementarity determining region 1 (CDR-H1), a CDR-H2, and a CDR-H3, respectively, comprising the amino acid sequences of a CDR-H1, a CDR-H2, and a CDR-H3 contained within the V_H region amino acid sequence set forth in SEQ ID NO: 1.

[0155] In some of any such embodiments, the V_H region contains a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 sequence having at least 90% sequence identity, respectively, to a FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the V_H region contains a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the V_H region contains a framework region 1 (FR1), a FR2, a FR3, and a FR4 sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and FR4 of the amino acid sequence set forth in SEQ ID NO: 1.

[0156] In some of any of such embodiments, the V_H region has the sequence of amino acids set forth in SEQ ID NO: 1.

[0157] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment is a heavy chain only, a V_H-only, and/or does not include a V_L or antigen-binding portion thereof and/or the antigen-binding site of the anti-idiotypic antibody or fragment includes residues from the heavy chain only and/or does not include residues from a light chain.

[0158] In some of any such embodiments, the anti-idiotypic antibody or fragment does not contain a light chain variable (V_L) region, does not contain a CDR-L1, CDR-L2, and/or CDR-L3, and/or is a single-domain antibody (sdAb) containing only the V_H region. In some embodiments, the antibody or fragment is a sdAb that only contains a V_H region from any as described.

[0159] In some embodiments of any of the anti-idiotypic antibodies or fragments containing any of the above V_H region sequences, the anti-idiotypic antibody or fragment further comprises a light chain variable (V_L) region. In some such embodiments, the V_L region has at least 90% sequence identity to the V_L region amino acid sequence set forth in SEQ ID NO: 5, such as at

least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_L region amino acid sequence set forth in SEQ ID NO: 5.

[0160] In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence set forth in SEQ ID NO: 14 or 87. In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence set forth in SEQ ID NO: 14 or 87.

[0161] In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 1 (CDR-L1) comprising the amino acid sequence set forth in SEQ ID NO: 12 or 85, and/or a CDR-L1 contained within the V_L sequence set forth in SEQ ID NO: 5; and/or a light chain complementarity determining region 2 (CDR-L2) comprising the amino acid sequence set forth in SEQ ID NO: 13 or 86, and/or a CDR-L2 contained within the V_L sequence set forth in SEQ ID NO: 5. In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence set forth in SEQ ID NO: 12 or 85, and/or a CDR-L1 contained within the V_L sequence set forth in SEQ ID NO: 5; and/or a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence set forth in SEQ ID NO: 13 or 86, and/or a CDR-L2 contained within the V_L sequence set forth in SEQ ID NO: 5.

[0162] In some of any such embodiments, the V_L region comprises a CDR-L1 containing the amino acid sequence set forth in SEQ ID NO: 12 or 85; a CDR-L2 containing the amino acid sequence set forth in SEQ ID NO: 13 or 86; and a CDR-L3 containing the amino acid sequence set forth in SEQ ID NO: 14 or 57.

[0163] In some of any such embodiments, the V_L region comprises the CDR-L1, CDR-L2, and CDR-L3, respectively, comprising the amino acid sequences of a CDR-L1, a CDR-L2, and a CDR-L3 contained within the V_L region amino acid sequence set forth in SEQ ID NO: 5.

[0164] In some of any such embodiments, the V_L region comprises a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 having at least 90% sequence identity, respectively, to the FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 5. In some embodiments, the V_L region comprises a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence

identity, respectively, to a FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 5. In some embodiments, the V_L region comprises a framework region 1 (FR1), a FR2, a FR3, and a FR4 sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and FR4 of the amino acid sequence set forth in SEQ ID NO: 5.

[0165] In some of any such embodiments, the V_L region has the amino acid sequence set forth in SEQ ID NO: 5.

[0166] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that comprise the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the V_H region amino acid sequence set forth in SEQ ID NO: 1; and/or comprise the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the light chain variable (V_L) region amino acid sequence set forth in SEQ ID NO: 5.

[0167] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include the V_H and V_L regions having amino acid sequences having at least 90 %, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NOs: 1 and 5, respectively.

[0168] In some embodiments, provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include the V_H and V_L regions having amino acid sequences set forth in SEQ ID NOs: 1 and 5, respectively.

[0169] In some of any such embodiments, the V_H and V_L regions include the amino acid sequences of SEQ ID NOs: 1 and 5, respectively.

[0170] In some embodiments, the anti-idiotypic antibody specific to antibody SJ25C1 or an antigen-binding fragment thereof is a single-chain antibody fragment, such as an scFv or diabody. In some embodiments, the single-chain antibody includes one or more linkers joining two antibody domains or regions, such as a variable heavy chain (V_H) region and a variable light chain (V_L). The linker typically is a peptide linker, e.g., a flexible and/or soluble peptide linker. Among the linkers are those rich in glycine and serine and/or in some cases threonine. In some embodiments, the linkers further include charged residues such as lysine and/or glutamate, which can improve solubility. In some embodiments, the linkers further include one or more proline.

[0171] In some embodiments, the anti-idiotypic antibody is an intact antibody or full-length antibody. In some embodiments, the anti-ID may contain at least a portion of an immunoglobulin constant region, such as one or more constant region domains. In some embodiments, the constant regions include a light chain constant region (CL) and/or a heavy chain constant region 1 (CH1). In some embodiments, the anti-ID includes a CH2 and/or CH3 domain, such as an Fc region. In some embodiments, the Fc region is an Fc region of a human IgG, such as IgG1 or IgG4. In some embodiments, the anti-idiotypic antibody contains the CH domain set forth in SEQ ID NO:2 or a portion thereof or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:2 or a portion thereof. In some embodiments, the anti-idiotypic antibody contains the CL domain set forth in SEQ ID NO:6 or a portion thereof or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:6 or a portion thereof.

[0172] In some embodiments, the anti-idiotypic antibody specific for SJ25C1 comprises the heavy chain sequence set forth in SEQ ID NO:3 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:3 and/or comprises the light chain sequence set forth in SEQ ID NO:7 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:7. In some embodiments, the anti-idiotypic antibody specific for SJ25C1 comprises the heavy chain sequence set forth in SEQ ID NO:3 and/or the light chain sequence set forth in SEQ ID NO:7. In some embodiments, the heavy chain and/or light chain of the anti-idiotypic antibody further comprises a signal peptide. In some cases, the signal peptide has the sequence set forth in SEQ ID NO:4 or SEQ ID NO: 8.

[0173] In some embodiments, the anti-idiotypic antibody is an antigen-binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, a single chain variable fragment (scFv) or a single domain antibody.

[0174] Accordingly, provided are single-chain antibody fragments, such as scFvs and diabodies, particularly human single-chain fragments, typically comprising linker(s) joining two anti-idiotypic antibody domains or regions, such as V_H and V_L domains. The linker typically is a peptide linker, e.g., a flexible and/or soluble peptide linker, such as one rich in glycine and serine.

[0175] In some aspects, the linkers rich in glycine and serine (and/or threonine) include at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% such amino acid(s). In some embodiments, they include at least at or about 50%, 55%, 60%, 70%, or 75%, glycine, serine, and/or threonine. In some embodiments, the linker is comprised substantially entirely of glycine, serine, and/or threonine. The linkers generally are between about 5 and about 50 amino acids in length, typically between at or about 10 and at or about 30, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and in some examples between 10 and 25 amino acids in length. Exemplary linkers include linkers having various numbers of repeats of the sequence GGGS (3GS; SEQ ID NO: 29) or GGGGS (4GS; SEQ ID NO: 26), such as between 2, 3, 4, and 5 repeats of such a sequence. Exemplary linkers include those having or consisting of a sequence set forth in SEQ ID NO: 25 (GGGGSGGGGSGGGGS). Exemplary linkers further include those having or consisting of the sequence set forth in SEQ ID NO: 33 (GSTSGSGKPGSGEGSTKG).

[0176] In some embodiments, the anti-idiotypic antibodies include isolated antibodies. In some embodiments, the anti-ID is humanized, recombinant, and/or monoclonal. In some embodiments, the anti-ID is human.

[0177] In some embodiments, the anti-idiotypic antibody specific for SJ25C1 is humanized. In particular embodiments, all or substantially all CDR amino acid residues of the humanized anti-idiotypic antibody specific for SJ25C1 are derived from anti SJ25C1 non-human CDRs. In some embodiments, the humanized anti-idiotypic antibody specific for SJ25C1 includes at least a portion of an antibody constant region derived from a human antibody.

[0178] In certain embodiments, the humanized anti-idiotypic antibody specific for SJ25C1 includes a human immunoglobulin (recipient antibody) in which residues from the heavy chain variable region of the recipient are replaced by residues from a heavy chain variable region of the nonhuman anti-idiotypic antibody specific for SJ25C1. In some instances, FR residues of the

human immunoglobulin are replaced by corresponding non-human residues. In some embodiments, the humanized antibody contains FR derived from different genes. In some embodiments, the humanized anti-idiotypic antibody specific for SJ25C1 contains at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

[0179] In some embodiments, the humanized anti-idiotypic antibody specific for SJ25C1 contains an altered human acceptor antibody variable domain sequences that have been rendered to encode one or more amino acid residues of position 4, 35, 38, 43, 44, 46, 58, 62, 64, 65, 66, 67, 68, 69, 73, 85, 98 (Kabat) of the light variable region and 2, 4, 36, 39, 43, 45, 69, 70, 74, 75, 76, 78, 92(Kabat) of the heavy variable region corresponding to the non-human donor sequence.

[0180] In certain embodiments, the anti-idiotypic antibody specific for SJ25C1 is humanized. In particular embodiments, the humanized anti-idiotypic antibody specific for SJ25C1 contains one or more of a CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 region of a non-human anti-idiotypic antibody that is specific for SJ25C1. In some embodiments, some or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 region of contain one or more amino acid modifications. In some embodiments, the modifications replacing a nonhuman amino acid residue with a human residue. In particular embodiments, the one or more of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 are inserted into the FR regions of a human antibody. In particular embodiments, the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody are the CDRs of the VH and VL regions having amino acid sequences set forth in SEQ ID NOs: 1 and 5, respectively. In some embodiments, all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the anti-idiotypic antibody specific for SJ25C1 are inserted into the FRs of the human antibody. In particular embodiments, the CDR and FR regions are the regions as identified by Kabat, Chothia, AbM, and/or and Contact schemes.

[0181] In particular embodiments, one or more or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody specific for SJ25C1 are inserted into framework regions of a human antibody. In certain embodiments, the human antibody is an IgA, IgD, IgE, IgG, and IgM antibody. In particular embodiments, the human antibody is one of a subclass of human IgA, IgD, IgE, IgG, and IgM, e.g., human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, or IgA₂. In some embodiments, one or more or all of the CDR-L1, CDR-L2,

CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody specific for SJ25C1 are inserted into framework regions of an antigen binding region that is from and/or is derived from a human antibody. In certain embodiments, the antigen binding fragment is from and/or is derived from a human IgA, IgD, IgE, IgG, and IgM antibody. The subunit structures and three-dimensional configurations of different classes of human immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). In some embodiments, the human antibody or antigen binding fragment thereof may be part of a larger fusion molecule, formed by covalent or non-covalent association of the human antibody with one or more other proteins or peptides.

[0182] In some embodiments, one or more or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody specific for SJ25C1 are inserted into framework regions of a human antibody or antigen-binding fragment thereof having all or a portion of an Fc region. In certain embodiments, the humanized anti-idiotypic antibody specific for SJ25C1 contains all or a portion of an Fc region. In some embodiments, the Fc region has one or more modifications, such as an amino acid modification (e.g. a substitution, insertion, or deletion) at one or more amino acid positions. Such modifications can be made, for example, to improve half-life, alter binding to one or more types of Fc receptors, and/or alter effector functions. In some embodiments, modified Fc regions have altered (e.g., decreased) binding to Fc α Rs, relative to that of an unmodified Fc region. In certain embodiments, the humanized anti-idiotypic antibody contains all or a portion of a modified Fc region having an altered (e.g., decreased) binding to Fc receptor relative to that of an unmodified Fc region. Non-limiting examples of Fc modifications that alter its binding to the Fc receptors are described, for example, in U.S. Pat. Nos. 7,217,797 and 7,732,570; and U.S. Application Nos. US 2010/0143254 and 2010/0143254.

[0183] In certain embodiments, a humanized anti-idiotypic antibody specific for SJ25C1 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 132 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 133. In particular embodiments, a humanized anti-idiotypic antibody specific for SJ25C1 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 134 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 135. In some embodiments, a humanized anti-idiotypic antibody

specific for SJ25C1 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 136 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 137.

B. FMC63-derived Antibodies

[0184] In some embodiments, provided are anti-idiotypic antibodies specific to a target anti-CD19 antibody that is or is derived from antibody FMC63 or an antigen-binding fragment thereof. In some embodiments, the provided antibodies or antigen-binding fragments are specific to an FMC63-derived scFv.

[0185] In some embodiments, provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include a heavy chain variable (V_H) region comprising at least 90% sequence identity to the V_H region amino acid sequence set forth in SEQ ID NO: 36 or 58, such as at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

[0186] In some embodiments, provided are antibodies or antigen-binding fragments thereof that include a V_H region having a heavy chain complementarity determining region 1 (CDR-H1) containing the amino acid sequence of $GYX_3FX_5X_6YX_8MX_{10}$ (SEQ ID NO: 108), wherein X_3 is T or S, X_5 is T or S, X_6 is D or R, X_8 is Y or W, and X_{10} is K or N; and/or a heavy chain complementarity determining region 2 (CDR-H2) containing the amino acid sequence of $WIGX_4IX_6PX_8X_9X_{10}X_{11}TX_{13}X_{14}NQX_{17}FKX_{20}$ (SEQ ID NO: 109), wherein X_4 is D or M, X_6 is N or H, X_8 is N or S, X_9 is N or D, X_{10} is G or S, X_{11} is G or E, X_{13} is D or R, X_{14} is Y or L, X_{17} is N or K, and X_{20} is G or D; and/or a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence of $AX_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ (SEQ ID NO: 110), wherein X_2 is R or S, X_3 is E or I, X_4 is G or Y, X_5 is N or Y, X_6 is N or E, X_7 is Y or null, X_8 is G or null, X_9 is S or null, X_{10} is R or null, X_{11} is D or null, X_{12} is A or null, X_{13} is M or null, X_{14} is D or E, and X_{15} is Y or A.

[0187] In some embodiments, provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include a heavy chain variable (V_H) region containing a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence set forth in SEQ ID NO: 46, 67, 94 or 104 and/or a CDR-H3 contained within the heavy chain variable (V_H) sequence set forth in SEQ ID NO: 36 or 58.

[0188] In some of any such embodiments, the V_H region includes a heavy chain complementarity determining region 1 (CDR-H1) comprising the amino acid sequence set forth in SEQ ID NO: 44, 65, 88, 89, 90, 98, 99, or 100, and/or a CDR-H1 contained within the V_H sequence set forth in SEQ ID NO: 36 or 58; and/or a heavy chain complementarity determining region 2 (CDR-H2) comprising the amino acid sequence set forth in SEQ ID NO: 45, 66, 91, 92, 93, 101, 102, or 103, and/or a CDR-H2 contained within the V_H sequence set forth in SEQ ID NO: 36 or 58.

[0189] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include a heavy chain variable (VH) region comprising a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and CDR-H3, wherein the CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 44, 65, 88, 89, 90, 98, 99, or 100; the CDR-H2 comprises the amino acid sequence set forth in SEQ ID NO: 45, 66, 91, 92, 93, 101, 102, or 103; and/or the CDR-H3 comprises the amino acid sequence set forth in SEQ ID NO: 46, 67, 94 or 104. In some embodiments, provided are antibodies or antigen-binding fragments thereof that include a CDR-H1 having the amino acid sequence set forth in SEQ ID NO: 44, 65, 88, 89, 90, 98, 99, or 100; a CDR-H2 having the amino acid sequence set forth in SEQ ID NO: 45, 66, 91, 92, 93, 101, 102, or 103; and a CDR-H3 having the amino acid sequence set forth in SEQ ID NO: 46, 67, 94 or 104.

[0190] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that includes a heavy chain complementarity determining region 1 (CDR-H1), a CDR-H2, and a CDR-H3, respectively, comprising the amino acid sequences of a CDR-H1, a CDR-H2, and a CDR-H3 contained within the V_H region amino acid sequence set forth in SEQ ID NO: 36 or 58.

[0191] In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof includes a CDR-H1 set forth in SEQ ID NOS: 44, 88, 89 or 90; a CDR-H2 set forth in SEQ ID NOS: 45, 91, 92 or 93; and/or a CDR-H3 set forth in SEQ ID NO: 46 or 94. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof includes a CDR-H1 set forth in SEQ ID NO: 65, 98, 99 or 100; a CDR-H2 set forth in SEQ ID NO: 66, 101, 102 or 103; and/or a CDR-H3 set forth in SEQ ID NO: 67 or 104, respectively.

[0192] In some of any such embodiments, the V_H region contains a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 sequence having at least 90% sequence identity, respectively, to a FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 36 or 58. In some embodiments, the V_H region contains a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 36 or 58. In some embodiments, the V_H region contains a framework region 1 (FR1), a FR2, a FR3, and a FR4 sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and FR4 of the amino acid sequence set forth in SEQ ID NO: 36 or 58.

[0193] In some of any of such embodiments, the V_H region has the sequence of amino acids set forth in SEQ ID NO: 36 or 58.

[0194] In some of any such embodiments, the anti-idiotypic antibody or antigen-binding fragment is a heavy chain only, a V_H-only, and/or does not include a V_L or antigen-binding portion thereof and/or the antigen-binding site of the anti-idiotypic antibody or fragment includes residues from the heavy chain only and/or does not include residues from a light chain.

[0195] In some of any such embodiments, the anti-idiotypic antibody or fragment does not contain a light chain variable (V_L) region, does not contain a CDR-L1, CDR-L2, and/or CDR-L3, and/or is a single-domain antibody (sdAb) containing only the V_H region. In some embodiments, the antibody or fragment is a sdAb that only contains a V_H region from any as described.

[0196] In some embodiments of any of the anti-idiotypic antibodies or fragments containing any of the above V_H region sequences, the anti-idiotypic antibody or fragment further comprises a light chain variable (V_L) region. In some such embodiments, the V_L region has at least 90% sequence identity to the V_L region amino acid sequence set forth in SEQ ID NO: 40 or 62, such as at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_L region amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0197] In some embodiments, provided are antibodies or antigen-binding fragments thereof that include a V_H region having a light chain complementarity determining region 1 (CDR-L1) containing the amino acid sequence of X₁AX₃X₄X₅X₆X₇X₈YX₁₀X₁₁WY (SEQ ID NO: 111),

wherein X_1 is S or R, X_3 is S or R, X_4 is S or G, X_5 is G or N, X_6 is V or I, X_7 is I or H, X_8 is N or null, X_{10} is M or L, and X_{11} is Y or A; and/or a light chain complementarity determining region 2 (CDR-L2) containing the amino acid sequence of $X_1X_2X_3YX_5X_6X_7X_8LAX_{11}$ (SEQ ID NO: 112), wherein X_1 is P or L, X_2 is W or L, X_3 is I or V, X_5 is L or N, X_6 is T or A, X_7 is S or K, X_8 is N or T, and X_{11} is S or D; and/or a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence of $QX_2X_3X_4X_5X_6PX_8T$ (SEQ ID NO: 113), wherein X_2 is Q or H, X_3 is W or F, X_4 is S or W, X_5 is S or W, X_6 is N or T, and X_8 is L or Y.

[0198] In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence set forth in SEQ ID NO: 49, 97, 70, or 107. In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence set forth in SEQ ID NO: 49, 97, 70, or 107.

[0199] In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 1 (CDR-L1) comprising the amino acid sequence set forth in SEQ ID NO: 47, 68, 95, or 105, and/or a CDR-L1 contained within the V_L sequence set forth in SEQ ID NO: 40 or 62; and/or a light chain complementarity determining region 2 (CDR-L2) comprising the amino acid sequence set forth in SEQ ID NO: 48, 69, 96, or 106, and/or a CDR-L2 contained within the V_L sequence set forth in SEQ ID NO: 40 or 62. In some of any such embodiments, the V_L region comprises a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence set forth in SEQ ID NO: 47, 68, 95, or 105, and/or a CDR-L1 contained within the V_L sequence set forth in SEQ ID NO: 40 or 62; and/or a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence set forth in SEQ ID NO: 48, 69, 96, or 106, and/or a CDR-L2 contained within the V_L sequence set forth in SEQ ID NO: 40 or 62.

[0200] In some of any such embodiments, the V_L region comprises a CDR-L1 containing the amino acid sequence set forth in SEQ ID NO: 47, 68, 95, or 105; a CDR-L2 containing the amino acid sequence set forth in SEQ ID NO: 48, 69, 96, or 106; and a CDR-L3 containing the amino acid sequence set forth in SEQ ID NO: 49, 97, 70, or 107.

[0201] In some of any such embodiments, the V_L region comprises the CDR-L1, CDR-L2, and CDR-L3, respectively, comprising the amino acid sequences of a CDR-L1, a CDR-L2, and a CDR-L3 contained within the V_L region amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0202] In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof includes a CDR-L1 set forth in SEQ ID NOS: 47 or 95; a CDR-L2 set forth in SEQ ID NOS: 48 or 96; and/or a CDR-L3 set forth in SEQ ID NO: 49 or 97. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof includes a CDR-L1 set forth in SEQ ID NO: 68 or 105; a CDR-L2 set forth in SEQ ID NO: 69 or 106; and/or a CDR-L3 set forth in SEQ ID NO: 70 or 107, respectively.

[0203] In some of any such embodiments, the V_L region comprises a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 having at least 90% sequence identity, respectively, to the FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 40 or 62. In some embodiments, the V_L region comprises a framework region 1 (FR1), a FR2, a FR3, and/or a FR4 sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and/or FR4 of the amino acid sequence set forth in SEQ ID NO: 40 or 62. In some embodiments, the V_L region comprises a framework region 1 (FR1), a FR2, a FR3, and a FR4 sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98%, 99% sequence identity, respectively, to a FR1, FR2, FR3, and FR4 of the amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0204] In some of any such embodiments, the V_L region has the amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0205] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that comprise the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the V_H region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or comprise the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the light chain variable (V_L) region amino acid sequence set forth in SEQ ID NO: 40 or 62.

[0206] Provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include the V_H region having amino acid sequences having at least 90 %, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NOS: 36 or 58 and V_L region having amino

acid sequences having at least 90 %, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NOs: 40 or 62.

[0207] In some embodiments, provided are anti-idiotypic antibodies or antigen-binding fragments thereof that include the V_H region having amino acid sequences set forth in SEQ ID NOs: 36 or 58 and V_L region having amino acid sequences set forth in SEQ ID NOs: 40 or 62. In some of embodiments, the provided antibody contains the V_H region set forth in SEQ ID NO: 36 and the V_L region set forth in SEQ ID NOs:40. In some of embodiments, the provided antibody contains the V_H region set forth in SEQ ID NO: 58 and the V_L region set forth in SEQ ID NOs: 62.

[0208] In some embodiments, the anti-idiotypic antibody is a single-chain antibody fragment, such as an scFv or diabody. In some embodiments, the single-chain antibody includes one or more linkers joining two antibody domains or regions, such as a variable heavy chain (V_H) region and a variable light chain (V_L). The linker typically is a peptide linker, e.g., a flexible and/or soluble peptide linker. Among the linkers are those rich in glycine and serine and/or in some cases threonine. In some embodiments, the linkers further include charged residues such as lysine and/or glutamate, which can improve solubility. In some embodiments, the linkers further include one or more proline.

[0209] In some embodiments, the anti-idiotypic antibody is an intact antibody or full-length antibody. In some embodiments, the anti-ID may contain at least a portion of an immunoglobulin constant region, such as one or more constant region domains. In some embodiments, the constant regions include a light chain constant region (CL) and/or a heavy chain constant region 1 (CH1). In some embodiments, the anti-ID includes a CH2 and/or CH3 domain, such as an Fc region. In some embodiments, the Fc region is an Fc region of a human IgG, such as IgG1 or IgG4. In some embodiments, the anti-idiotypic antibody contains the CH domain set forth in SEQ ID NO:37 or 59 or a portion thereof or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:37 or 59 or a portion thereof. In some embodiments, the anti-idiotypic antibody contains the CL domain set forth in SEQ ID NO:41 or 63 or a portion thereof or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:41 or 63 or a portion thereof.

[0210] In some embodiments, the anti-idiotypic antibody specific for FMC63 comprises the heavy chain sequence set forth in SEQ ID NO:38 or 60 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:38 or 60 and/or comprises the light chain sequence set forth in SEQ ID NO:42 or 63 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:42 or 63. In some embodiments, the anti-idiotypic antibody specific for FMC63 comprises the heavy chain sequence set forth in SEQ ID NO:38 and/or the light chain sequence set forth in SEQ ID NO:42. In some embodiments, the anti-idiotypic antibody specific for FMC63 comprises the heavy chain sequence set forth in SEQ ID NO:60 and/or the light chain sequence set forth in SEQ ID NO:63. In some embodiments, the heavy chain and/or light chain of the anti-idiotypic antibody further comprises a signal peptide. In some cases, the signal peptide has the sequence set forth in SEQ ID NO:39, 43, 61 or 64.

[0211] In some embodiments, the anti-idiotypic antibody is an antigen-binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, a single chain variable fragment (scFv) or a single domain antibody.

[0212] Accordingly, provided are single-chain antibody fragments, such as scFvs and diabodies, particularly human single-chain fragments, typically comprising linker(s) joining two anti-idiotypic antibody domains or regions, such as V_H and V_L domains. The linker typically is a peptide linker, e.g., a flexible and/or soluble peptide linker, such as one rich in glycine and serine.

[0213] In some aspects, the linkers rich in glycine and serine (and/or threonine) include at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% such amino acid(s). In some embodiments, they include at least at or about 50%, 55%, 60%, 70%, or 75%, glycine, serine, and/or threonine. In some embodiments, the linker is comprised substantially entirely of glycine, serine, and/or threonine. The linkers generally are between about 5 and about 50 amino acids in length, typically between at or about 10 and at or about 30, e.g., 10, 11, 12, 13, 14, 15, 16, 17,

18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and in some examples between 10 and 25 amino acids in length. Exemplary linkers include linkers having various numbers of repeats of the sequence GGS (3GS; SEQ ID NO: 29) or GGGGS (4GS; SEQ ID NO: 26), such as between 2, 3, 4, and 5 repeats of such a sequence. Exemplary linkers include those having or consisting of a sequence set forth in SEQ ID NO: 25 (GGGGSGGGGSGGGGS). Exemplary linkers further include those having or consisting of the sequence set forth in SEQ ID NO: 33 (GSTSGSGKPGSGEGSTKG).

[0214] In some embodiments, the anti-idiotypic antibodies include isolated antibodies. In some embodiments, the anti-ID is humanized, recombinant, and/or monoclonal. In some embodiments, the anti-ID is human.

[0215] In some embodiments, the anti-idiotypic antibody specific for FMC63 is humanized. In particular embodiments, all or substantially all CDR amino acid residues of the humanized anti-idiotypic antibody specific for FMC63 are derived from non-human, anti-FMC63 CDRs. In some embodiments, the humanized anti-idiotypic antibody specific for FMC63 includes at least a portion of an antibody constant region derived from a human antibody.

[0216] In certain embodiments, the humanized anti-idiotypic antibody specific for FMC63 includes a human immunoglobulin (recipient antibody) in which residues from the heavy chain variable region of the recipient are replaced by residues from a heavy chain variable region of the nonhuman anti-idiotypic antibody specific for FMC63. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. In some embodiments, the humanized antibody contains FR derived from different genes. In some embodiments, the humanized anti-idiotypic antibody specific for FMC63 contains at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

[0217] In some embodiments, the humanized anti-idiotypic antibody specific for FMC63 contains an altered human acceptor antibody variable domain sequences that have been rendered to encode one or more amino acid residues of position 4, 35, 38, 43, 44, 46, 58, 62, 64, 65, 66, 67, 68, 69, 73, 85, 98 (Kabat) of the light variable region and 2, 4, 36, 39, 43, 45, 69, 70, 74, 75, 76, 78, 92(Kabat) of the heavy variable region corresponding to the non-human donor sequence .

[0218] In certain embodiments, the anti-idiotypic antibody specific for FMC63 is humanized. In particular embodiments, the humanized anti-idiotypic antibody specific for FMC63 contains one or more of a CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of a non-human anti-idiotypic antibody specific for FMC63. In some embodiments, some or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 region of contain one or more amino acid modifications. In some embodiments, the modifications replacing a nonhuman amino acid residue with a human residue. In particular embodiments, the one or more of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 are inserted into human FR regions. In particular embodiments, the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody are the CDRs of the VH regions having amino acid sequences set forth in SEQ ID NOs: 36 or 58. In certain embodiments, the CDRs of the nonhuman anti-idiotypic VH region are or include a CDR-H1 containing with the amino acid sequence set forth in SEQ ID NO: 44, 65, 88, 89, 90, 98, 99, or 100; the CDR-H2 containing the amino acid sequence set forth in SEQ ID NO: 45, 66, 91, 92, 93, 101, 102, or 103; and/or the CDR-H3 containing the amino acid sequence set forth in SEQ ID NO: 46, 67, 94 or 104. In some embodiments, the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody are the CDRs of the VL regions having amino acid sequences set forth in SEQ ID NOs: 40 or 62. In some embodiments, the CDRs of the nonhuman anti-idiotypic VL region are or include a CDR-L1 containing the amino acid sequence set forth in SEQ ID NO: 47, 68, 95, or 105; a CDR-L2 containing the amino acid sequence set forth in SEQ ID NO: 48, 69, 96, or 106; and a CDR-L3 containing the amino acid sequence set forth in SEQ ID NO: 49, 97, 70, or 107. In some embodiments, all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 regions of the anti-idiotypic antibody specific for FMC63 are inserted into the FRs of the human antibody. In particular embodiments, the CDR and FR regions are the regions as identified by Kabat, Chothia, AbM, and/or and Contact schemes.

[0219] In particular embodiments, one or more or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody specific for FMC63 are inserted into framework regions of a human antibody. In certain embodiments, the human antibody is an IgA, IgD, IgE, IgG, and IgM antibody. In particular embodiments, the human antibody is one of a subclass of human IgA, IgD, IgE, IgG, and IgM, e.g., human IgG₁, IgG₂,

IgG₃, IgG₄, IgA₁, or IgA₂. In some embodiments, one or more or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody specific for FMC63 are inserted into framework regions of an antigen binding region that is from and/or is derived from a human antibody. In certain embodiments, the antigen binding fragment is from and/or is derived from a human IgA, IgD, IgE, IgG, and IgM antibody. The subunit structures and three-dimensional configurations of different classes of human immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). In some embodiments, the human antibody or antigen binding fragment thereof may be part of a larger fusion molecule, formed by covalent or non-covalent association of the human antibody with one or more other proteins or peptides.

[0220] In some embodiments, one or more or all of the CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 of the nonhuman anti-idiotypic antibody specific for FMC63 are inserted into framework regions of a human antibody or antigen-binding fragment thereof having all or a portion of an Fc region. In certain embodiments, the humanized anti-idiotypic antibody specific for FMC63 contains all or a portion of an Fc region. In some embodiments, the Fc region has one or more modifications, such as an amino acid modification (e.g. a substitution, insertion, or deletion) at one or more amino acid positions. Such modifications can be made, for example, to improve half-life, alter binding to one or more types of Fc receptors, and/or alter effector functions. In some embodiments, modified Fc regions have altered (e.g., decreased) binding to Fc α R_s, relative to that of an unmodified Fc region. In certain embodiments, the humanized anti-idiotypic antibody contains all or a portion of a modified Fc region having an altered (e.g., decreased) binding to Fc receptor relative to that of an unmodified Fc region. Non-limiting examples of Fc modifications that alter its binding to the Fc receptors are described, for example, in U.S. Pat. Nos. 7,217,797 and 7,732,570; and U.S. Application Nos. US 2010/0143254 and 2010/0143254.

[0221] In certain embodiments, a humanized anti-idiotypic antibody specific for FMC63 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 138 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 139. In particular embodiments, a humanized anti-idiotypic antibody specific for FMC63 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 140 and a light chain containing the amino acid sequence

set forth in SEQ ID NO: 141. In some embodiments, a humanized anti-idiotypic antibody specific for FMC63 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 142 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 143. In certain embodiments, a humanized anti-idiotypic antibody specific for FMC63 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 144 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 145. In particular embodiments, a humanized anti-idiotypic antibody specific for FMC63 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 146 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 147. In some embodiments, a humanized anti-idiotypic antibody specific for FMC63 has a heavy chain containing the amino acid sequence set forth in SEQ ID NO: 148 and a light chain containing the amino acid sequence set forth in SEQ ID NO: 149.

C. Exemplary Features

[0222] Anti-idiotypic antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various known assays. In one aspect, the anti-idiotypic antibody is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blotting, and/or flow cytometric assays, including cell-based binding assays, for example, assessing binding of the anti-idiotypic antibody (e.g., conjugated to a fluorescent marker or tagged) to a cell presenting the target anti-CD19 antibody moiety, in some cases compared to results using cells that do not express the target anti-CD19 antibody moiety. Binding affinity may be measured as K_d or EC_{50} .

[0223] In some embodiments of any of the provided antibodies, e.g. any of the provided antibodies in Section A above, the target anti-CD19 antibody moiety is SJ25C1 or is an antibody derived from SJ25C1.

[0224] In some embodiments of any of the provided antibodies, e.g. any of the provided antibodies in Section B above, the target anti-CD19 antibody moiety is FMC63 or is an antibody derived from FMC63.

[0225] Competition assays may be used to identify an antibody that competes with any of the anti-idiotypic antibodies described herein. Assays for mapping epitopes bound by the anti-idiotypic antibodies and reference antibodies also may be used and are known.

[0226] In some embodiments, the anti-idiotypic antibody does not cross-react with an anti-CD19 antibody moiety different from the target anti-CD19 antibody moiety. In some embodiments, the target anti-CD19 antibody moiety is derived from the SJ25C1 antibody. In some embodiments, the target anti-CD19 antibody moiety is derived from the SJ25C1 antibody, and the anti-idiotypic antibody does not cross-react with an anti-CD19 antibody moiety derived from the FMC63 antibody, an anti-CD19 antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 30 and a V_L comprising the amino acid sequence of SEQ ID NO: 31. In some embodiments, the target anti-CD19 antibody moiety is derived from the FMC63 antibody. In some embodiments, the target anti-CD19 antibody moiety is derived from the FMC63 antibody, and the anti-idiotypic antibody does not cross-react with an anti-CD19 antibody moiety derived from the SJ25C1 antibody, an anti-CD19 antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 23 and a V_L comprising the amino acid sequence of SEQ ID NO: 24.

[0227] In some embodiments, the anti-idiotypic antibody specifically binds to a target anti-CD19 antibody moiety that is part of a fusion protein, such as a recombinant receptor. In some embodiments, the anti-idiotypic antibody does not bind to any epitope in the fusion protein outside of the target anti-CD19 antibody moiety. For example, in some embodiments, the target anti-CD19 antibody moiety is, or is part of, the antigen-binding domain of a chimeric antigen receptor (CAR), and the anti-idiotypic antibody does not bind any epitope outside of the antigen-binding domain. In some embodiments, the CAR antigen-binding domain comprises or consists of an scFv.

[0228] In some embodiments, the anti-idiotypic antibody specifically binds to a target anti-CD19 antibody moiety that is an scFv contained in a CAR. In some embodiments, the anti-idiotypic antibody specifically binds to an epitope overlapping one or more complementarity determining regions (CDRs) of the target anti-CD19 scFv. In some embodiments, the anti-idiotypic antibody does not bind any epitopes in the CAR outside of the scFv; in some embodiments, it does not bind to a reference antibody. In some embodiments, the reference antibody specifically binds to the same antigen as the target antibody, e.g., to the CD19 and/or comprises one or more variable heavy and/or variable light framework region(s) having at least 90, 95, 96, 97, 98, or 99 % identity to the corresponding framework region(s) of the target

antibody (in some aspects, the one or more framework regions comprise an FR1, FR2, FR3, and/or FR4 of the heavy and/or the light chain); and/or contains the same heavy and/or light chain v-gene (or v-gene usage) as the target antibody and/or is derived from the same v-gene sequence as the target antibody. In some aspects, the reference antibody is FMC63. In some aspects, the reference antibody is SJ25C1. In some embodiments, the CAR comprises a spacer linking the scFv to its transmembrane domain, and the anti-idiotypic antibody does not bind any epitope in the spacer. In some embodiments, the spacer is a sequence derived from CD28, such as an extracellular portion from CD28. In some embodiments, the spacer comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the anti-idiotypic antibody does not bind any epitope in an Fc domain, such as the Fc domain of IgG1. In some embodiments, the Fc domain is an IgG1 Fc domain lacking the hinge region. In some embodiments, the Fc domain comprises the amino acid sequence of SEQ ID NO: 32.

[0229] In some embodiments, the anti-idiotypic antibody does not cross-react with a different CAR. In some embodiments, the anti-idiotypic antibody does not cross-react with a different anti-CD19 CAR. In some embodiments, the anti-idiotypic antibody does not cross-react with an anti-CD19 antibody moiety, e.g., of a reference antibody, having one or more different idiotopes compared to the target anti-CD19 scFv. In some embodiments, the anti-idiotypic antibody is specific for a target anti-CD19 scFv of a CAR derived from the SJ25C1 antibody. In some embodiments, the target anti-CD19 antibody moiety is derived from the SJ25C1 antibody, and the anti-idiotypic antibody does not cross-react with a CAR containing an anti-CD19 antibody moiety derived from the FMC63 antibody. In some embodiments, the anti-idiotypic antibody is specific for a target anti-CD19 scFv of a CAR derived from the FMC63 antibody. In some embodiments, the target anti-CD19 antibody moiety is derived from the FMC63 antibody, and the anti-idiotypic antibody does not cross-react with a CAR containing anti-CD19 antibody moiety derived from the SJ25C1 antibody.

[0230] In some embodiments, the anti-idiotypic antibody is an agonist of the CAR. In some embodiments, the anti-idiotypic antibody is an antagonist of the CAR.

[0231] In some embodiments, the provided anti-idiotypic antibodies are capable of binding a target anti-CD19 moiety, such as antibody SJ25C1 or FMC63, with at least a certain affinity, as measured by any of a number of known methods. In some embodiments, the affinity is

represented by an equilibrium dissociation constant (K_D); in some embodiments, the affinity is represented by EC_{50} . In certain embodiments, the binding affinity (EC_{50}) and/or the dissociation constant of the anti-idiotypic antibody to the anti-CD19 moiety is at or about or less than at or about 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nM, such as between at or about 1 nM and at or about 15 nM, e.g., between at or about 5 and at or about 10 nM. In one embodiment, the extent of binding of an anti-idiotypic antibody to a moiety unrelated to the target anti-CD19 moiety is less than, at, or about 10% of the binding of the antibody to the target anti-CD19 moiety as measured, e.g., by a radioimmunoassay (RIA).

D. Nucleic Acids

[0232] Also provided are nucleic acids encoding the antibodies and/or portions, e.g., chains, thereof. Among the provided nucleic acids are those encoding the anti-idiotypic antibodies described herein. The nucleic acids may include those encompassing natural and/or non-naturally occurring nucleotides and bases, e.g., including those with backbone modifications. The terms “nucleic acid molecule”, “nucleic acid” and “polynucleotide” may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. “Nucleic acid sequence” refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide. Exemplary nucleic acids and vectors are those having the sequences set forth as SEQ ID NOs: 15-22, 50-57, 71-77 and CDR-encoding portions thereof, as well as sequences containing at least at or about 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity thereto. The nucleic acid may encode an amino acid sequence comprising the V_L and/or an amino acid sequence comprising the V_H of the anti-idiotypic antibody (e.g., the light and/or heavy chains of the antibody).

[0233] Also provided are vectors containing the nucleic acids, host cells containing the vectors, e.g., for producing the antibodies. Also provided are methods for producing the antibodies. In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising

the V_L of the antibody and an amino acid sequence comprising the V_H of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H of the antibody. In some embodiments, a method of making the anti-idiotypic antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

E. Methods of Producing the Antibodies

[0234] Also provided are methods of making an anti-idiotypic antibody, such as of any of the provided embodiments. In some embodiments, for recombinant production of the anti-idiotypic antibody, nucleic acid encoding an antibody, e.g., as described above, may be isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0235] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been modified to mimic or approximate those in human cells, resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22: 1409-1414 (2004), and Li et al., *Nat. Biotech.* 24: 210-215 (2006).

[0236] Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44, Lec13 CHO cells, and FUT8 CHO cells; PER.C6[®] cells; and NSO cells. In some embodiments, the antibody heavy chains and/or light chains may be expressed in yeast. See, e.g., U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or light chains. For example, in some embodiments, CHO

cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0237] In some embodiments, the anti-idiotypic antibody is produced in a cell-free system. Exemplary cell-free systems are described, *e.g.*, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

[0238] The provided embodiments further include vectors and host cells and other expression systems for expressing and producing the antibodies and other binding proteins, including eukaryotic and prokaryotic host cells, including bacteria, filamentous fungi, and yeast, as well as mammalian cells such as human cells, as well as cell-free expression systems.

[0239] The anti-idiotypic antibodies or antibody moieties can be humanized antibodies or human antibodies. A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0240] Among the provided anti-idiotypic antibodies or antibody moieties are human antibodies. A “human antibody” is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human.

[0241] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

F. Immunoconjugates

[0242] In some embodiments, the anti-idiotypic antibody is or is part of an immunoconjugate (anti-idiotypic antibody immunoconjugate), in which the anti-idiotypic antibody is conjugated to one or more heterologous molecule(s), such as, but not limited to, a cytotoxic or an imaging agent. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); chemotherapeutic agents (e.g., maytansinoids, taxanes, methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins. In some embodiments, the antibody is conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0243] Among the anti-idiotypic antibody immunoconjugates are antibody-drug conjugates (ADCs), in which an anti-idiotypic antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman *et al.*, *Cancer*

Res. 53: 3336-3342 (1993); and Lode *et al.*, *Cancer Res.* 58: 2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz *et al.*, *Current Med. Chem.* 13: 477-523 (2006); Jeffrey *et al.*, *Bioorganic & Med. Chem. Letters* 16: 358-362 (2006); Torgov *et al.*, *Bioconj. Chem.* 16: 717-721 (2005); Nagy *et al.*, *Proc. Natl. Acad. Sci. USA* 97: 829-834 (2000); Dubowchik *et al.*, *Bioorg. & Med. Chem. Letters* 12: 1529-1532 (2002); King *et al.*, *J. Med. Chem.* 45: 4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0244] Also among the anti-idiotypic antibody immunoconjugates are those in which the antibody is conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0245] Also among the anti-idiotypic antibody immunoconjugates are those in which the anti-idiotypic antibody is conjugated to a radioactive atom to form a radioconjugate. Exemplary radioactive isotopes include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu.

[0246] Conjugates of an anti-idiotypic antibody and cytotoxic agent may be made using any of a number of known protein coupling agents, e.g., linkers, (see Vitetta *et al.*, *Science* 238: 1098 (1987)), WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell, such as acid-labile linkers, peptidase-sensitive linkers, photolabile linkers, dimethyl linkers, and disulfide-containing linkers (Chari *et al.*, *Cancer Res.* 52: 127-131 (1992); U.S. Patent No. 5,208,020).

[0247] Also provided are anti-idiotypic antibody immunoconjugates comprising an anti-idiotypic antibody attached to a label, e.g., a detectable label, which can generate a detectable signal, indirectly or directly. These anti-idiotypic antibody immunoconjugates can be used for research or diagnostic applications. The label is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as ³H, ¹⁴C, ³²P, ³⁵S, ¹²³I, ¹²⁵I, ¹³¹I; a fluorescent (fluorophore) or chemiluminescent

(chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, β -galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. In some embodiments, the label is a radioactive atom for scintigraphic studies, for example ^{99}Tc or ^{123}I , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, *e.g.*, for PET imaging (WO 2011/056983).

[0248] Examples of detectable labels include but are not limited to radionucleotides, enzymes, coenzymes, fluorescers, chemilumescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin, coumarin, Alexa488, Oregon green 488, rhodamine green, Alexa 532, Cy3, Bodipy 588/586, Alexa586, TAMRA, Rox, Alexa 594, Texas red, Bodipy 630/650, Cy5, Alexa647, IR Dye 680, IR Dye 680, IR Dye 700 DX, Cy5.5, Alexa 750, IR Dye 800CW, IR Dye 800, Atto 532, and Atto 465.

[0249] In some embodiments, the anti-idiotypic antibody immunoconjugate is detectable indirectly. For example, a secondary antibody that is specific for the anti-idiotypic antibody immunoconjugate and contains a detectable label can be used to detect the anti-idiotypic antibody immunoconjugate.

Multispecific Antibodies

[0250] In certain embodiments, the anti-idiotypic antibodies are multispecific. Among the multispecific binding molecules are multispecific antibodies, including, *e.g.* bispecific. Multispecific binding partners, *e.g.*, antibodies, have binding specificities for at least two different sites, which may be in the same or different antigens. In certain embodiments, one of the binding specificities is for an anti-CD19 antibody moiety and the other is for another antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of an

anti-CD19 antibody moiety. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express an anti-CD19 antibody moiety on their surface, such as anti-CD19 CAR T cells. Bispecific antibodies can be prepared as full length antibodies or antibody fragments. Among the bispecific antibodies are multispecific single-chain antibodies, e.g., diabodies, triabodies, and tetrabodies, tandem di-scFvs, and tandem tri-scFvs.

G. Variants

[0251] In certain embodiments, the anti-idiotypic antibodies include one or more amino acid variations, e.g., substitutions, deletions, insertions, and/or mutations, compared to the sequence of an anti-idiotypic antibody described herein. Exemplary variants include those designed to improve the binding affinity and/or other biological properties of the anti-idiotypic antibody. Amino acid sequence variants of an anti-idiotypic antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the anti-idiotypic antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the anti-idiotypic antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

[0252] In certain embodiments, the anti-idiotypic antibodies include one or more amino acid substitutions, e.g., as compared to an anti-idiotypic antibody sequence described herein. Sites of interest for substitutional mutagenesis include the CDRs and FRs. Amino acid substitutions may be introduced into an anti-idiotypic antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, improved half-life, and/or improved effector function, such as the ability to promote antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). In some embodiments, the variant anti-idiotypic antibody exhibits retained or improved binding to a target anti-CD19 antibody or fragment thereof. For example, in some embodiments, the variant anti-idiotypic antibody exhibits an increase in binding affinity to the target anti-CD19 antibody of at least about 10% (such as at least about any of 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000% or more) as compared to the unmodified anti-idiotypic antibody.

[0253] In some embodiments, one or more residues within a CDR of a parent antibody (e.g. a humanized or human antibody) is/are substituted. In some embodiments, the substitution is made to revert a sequence or position in the sequence to a germline sequence, such as an antibody sequence found in the germline (e.g., human germline), for example, to reduce the likelihood of immunogenicity, e.g., upon administration to a human individual.

[0254] In some embodiments, alterations are made in CDR “hotspots,” residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207: 179-196 (2008)), and/or residues that contact antigen, with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178: 1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001)). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0255] In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. Such alterations may, for example, be outside of antigen contacting residues in the CDRs. In certain embodiments of the variant V_H and V_L sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0256] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants

of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

Modifications

[0257] In certain embodiments, the antibody is altered to increase or decrease the extent to which the antibody is glycosylated, for example, by removing or inserting one or more glycosylation sites by altering the amino acid sequence and/or by modifying the oligosaccharide(s) attached to the glycosylation sites, e.g., using certain cell lines.

[0258] Exemplary modifications, variants, and cell lines are described, e.g., in Patent Publication Nos. US 2003/0157108, US 2004/0093621, US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336: 1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Ripka et al. Arch. Biochem. Biophys. 249: 533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4): 680-688 (2006); and WO2003/085107); WO 2003/011878 (Jean-Mairet et al.); U.S. Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.); WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0259] Among the modified antibodies are those having one or more amino acid modifications in the Fc region, such as those having a human Fc region sequence or other portion of a constant region (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0260] Such modifications can be made, e.g., to improve half-life, alter binding to one or more types of Fc receptors, and/or alter effector functions.

[0261] Also among the variants are cysteine engineered antibodies such as “thioMAbs” and other cysteine engineered variants, in which one or more residues of an antibody are substituted with cysteine residues, in order to generate reactive thiol groups at accessible sites, e.g., for use in conjugation of agents and linker-agents, to produce immunoconjugates. Cysteine engineered antibodies are described, e.g., in U.S. Patent Nos. 7,855,275 and 7,521,541.

[0262] In some embodiments, the antibodies are modified to contain additional nonproteinaceous moieties, including water soluble polymers. Exemplary polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

II. METHODS OF IDENTIFYING ANTI-IDIOTYPE ANTIBODIES

[0263] In some embodiments, there is provided a method of identifying an anti-idiotypic antibody or antigen-binding fragment that specifically binds to a target antibody or antigen-binding fragment thereof, such as by using hybridoma methods with an immunogen comprising the target antibody or fragment thereof (*see* for example Kohler and Milstein, *Nature*, 256: 495 (1975) and Sergeeva *et al.*, *Blood*, 117(16): 4262-4272). The immunogen may comprise an immunogenicity-enhancing moiety fused to the target antibody or fragment. Such immunogenicity-enhancing moiety may have properties that include, without limitation, increasing solubility and half-life of the immunogen. Exemplary immunogenicity-enhancing moieties include Fc domains or fragments thereof. In some embodiments, the immunogenicity-enhancing moiety is an Fc domain (such as from IgG1, optionally human). In some embodiments, the immunogenicity-enhancing moiety is an Fc domain (such as from IgG1, optionally human) lacking all or a portion of the hinge region.

[0264] In some embodiments, there is provided a method of identifying an anti-idiotypic antibody or antigen-binding fragment that specifically binds to a target antibody or antigen-binding fragment thereof, comprising: (a) introducing into a subject a soluble immunogen comprising an antigen-binding fragment of the target antibody fused to an immunogenicity-enhancing moiety; and (b) identifying an antibody from the subject that specifically binds to the target antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding fragment comprises the variable heavy chain region and/or variable light chain region of the target antibody. In some embodiments, the antigen-binding fragment is a single chain fragment. In some embodiments, the antigen-binding fragment is an scFv. In some embodiments, the antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR).

[0265] In some embodiments, the immunogenicity-enhancing moiety is an Fc domain or fragment thereof, which optionally is a human IgG1 Fc. In some embodiments, the immunogenicity-enhancing moiety is an Fc domain lacking the hinge region. In some embodiments, the immunogenicity-enhancing moiety comprises the amino acid sequence set forth in SEQ ID NO: 32.

[0266] In some embodiments, identifying the antibody comprises using hybridoma techniques to identify individual antibody clones produced by the subject and screening the clones for binding to the target antibody or antigen-binding fragment thereof. In some embodiments, identifying the antibody comprises: (i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas; (ii) screening the hybridomas for production of antibodies that specifically bind the target antibody or the antigen-binding fragment thereof or a chimeric antigen receptor comprising the antigen-binding fragment; and (iii) sequencing an antibody from a hybridoma producing an antibody that specifically binds the target antibody or antigen-binding fragment, thereby identifying the anti-idiotypic antibody. In some embodiments, screening a hybridoma comprises determining the binding affinity of the hybridoma antibody for a target molecule comprising the target antibody or an idiotope of the target antibody, such as an scFv or a CAR or fragment thereof comprising the variable heavy chain region and/or variable light chain region of the target antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions. In some

embodiments, screening a hybridoma further comprises determining the binding affinity of the hybridoma antibody for a non-target molecule that does not comprise an idiotope of the target antibody, such as an Fc or fragment thereof or another antibody or fragment thereof that do not comprise the variable heavy chain region and/or variable light chain region of the target antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions, wherein binding of the hybridoma antibody to the target molecule but not the non-target molecule indicates that the hybridoma antibody specifically binds the target antibody.

[0267] In some of any such embodiments, the method comprises: (a) introducing into a subject a soluble immunogen comprising an scFv of the target antibody fused to an Fc domain or fragment thereof; and (b) identifying an antibody from the subject that specifically binds to a molecule comprising an idiotope of the target antibody, such as the immunogen. In some embodiments, the scFv is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR). In some embodiments, the Fc domain or fragment thereof is a human IgG1 Fc or fragment thereof. In some embodiments, the Fc domain or fragment thereof is an Fc domain lacking the hinge region. In some embodiments, the Fc domain or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO: 32. In some embodiments, identifying the antibody comprises using hybridoma techniques to identify individual antibody clones produced by the subject and screening the antibody clones for binding to a molecule comprising an idiotope of the target antibody, such as the immunogen. In some embodiments, identifying the antibody comprises: (i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas; (ii) screening the hybridomas for production of antibodies that specifically bind a molecule comprising an idiotope of the target antibody, such as the immunogen; and (iii) sequencing an antibody from a hybridoma producing an antibody that specifically binds a molecule comprising an idiotope of the target antibody, such as the immunogen, thereby identifying the anti-idiotypic antibody. In some embodiments, screening a hybridoma comprises determining the binding affinity of the hybridoma antibody for a target molecule comprising an idiotope of the target antibody, such as the immunogen, an scFv, or a CAR or fragment thereof comprising the variable heavy chain region and/or variable light chain region of the target antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions. In some embodiments, screening a

hybridoma further comprises determining the binding affinity of the hybridoma antibody for a non-target molecule that does not comprise an idiotope of the target antibody, such as an Fc or fragment thereof or another antibody or fragment thereof that does not comprise the variable heavy chain region and/or variable light chain region of the target antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions, wherein binding of the hybridoma antibody to the target molecule but not the non-target molecule indicates that the hybridoma antibody specifically binds the target antibody.

[0268] In some of any such embodiments, the method comprises: (a) introducing into a subject a soluble immunogen comprising an scFv of the target antibody fused to an Fc domain or fragment thereof; and (b) identifying an antibody from the subject that (i) binds to a target molecule comprising an idiotope of the target antibody, such as the immunogen, an scFv, or a CAR or fragment thereof comprising the variable heavy chain region and/or variable light chain region of the target antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions; and (ii) does not bind to a non-target molecule that does not comprise an idiotope of the target antibody, such as an Fc or fragment thereof or another antibody or fragment thereof that does not comprise the variable heavy chain region and/or variable light chain region of the target antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions. In some embodiments, the scFv is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR). In some embodiments, the Fc domain or fragment thereof is a human IgG1 Fc or fragment thereof. In some embodiments, the Fc domain or fragment thereof is an Fc domain lacking the hinge region. In some embodiments, the Fc domain or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO: 32. In some embodiments, identifying the antibody comprises using hybridoma techniques to identify individual antibody clones produced by the subject and screening the antibody clones for binding to the target and non-target molecules. In some embodiments, identifying the antibody comprises: (i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas; (ii) screening the hybridomas for production of antibodies that bind to the target molecule but not to the non-target molecule; and (iii) sequencing an antibody from a hybridoma producing an antibody that binds to the target molecule but not to the non-target molecule, thereby identifying the anti-idiotypic

antibody. In some embodiments, screening a hybridoma comprises determining the binding affinity of the hybridoma antibody for the target molecule and the non-target molecule.

[0269] In some of any such embodiments, the method comprises: (a) introducing into a subject a soluble immunogen comprising an scFv derived from the FMC63 antibody (such as an scFv comprising the amino acid sequence of SEQ ID NO: 34) fused to an Fc domain or fragment thereof; and (b) identifying an antibody from the subject that (i) binds to a target molecule comprising an idiotope of the FMC63 antibody, such as the immunogen, an scFv, or a CAR or fragment thereof comprising the variable heavy chain region and/or variable light chain region of the FMC63 antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions; and (ii) does not bind to a non-target molecule that does not comprise an idiotope of the FMC63 antibody, such as an Fc or fragment thereof or another antibody or fragment thereof that does not comprise the variable heavy chain region and/or variable light chain region of the FMC63 antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions. In some embodiments, the immunogen comprises the amino acid sequence set forth in SEQ ID NO:34 and SEQ ID NO:32, optionally separated by a linker, e.g. set forth in SEQ ID NO:33. In some embodiments, the immunogen comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, the antibody identified in (b) binds to the immunogen and does not bind to either an Fc domain or fragment thereof or a molecule comprising an scFv derived from the SJ25C1 antibody (such as an scFv comprising the amino acid sequence of SEQ ID NO: 28). In some embodiments, the antibody identified in (b) binds to a target molecule comprising the amino acid sequence of SEQ ID NO: 34 or 35 and does not bind to either a non-target molecule comprising the amino acid sequence of SEQ ID NO: 32 or a non-target molecule comprising the amino acid sequence of SEQ ID NO: 28.

[0270] In some of any such embodiments, the method comprises: (a) introducing into a subject a soluble immunogen comprising an scFv derived from the SJ25C1 antibody (such as an scFv comprising the amino acid sequence of SEQ ID NO: 28) fused to an Fc domain or fragment thereof; and (b) identifying an antibody from the subject that (i) binds to a target molecule comprising an idiotope of the SJ25C1 antibody, such as the immunogen, an scFv, or a CAR or fragment thereof comprising the variable heavy chain region and/or variable light chain region of the SJ25C1 antibody, or portions thereof, such as one or more of the CDRs of the VH

and/or VL regions; and (ii) does not bind to a non-target molecule that does not comprise an idiotope of the SJ25C1 antibody, such as an Fc or fragment thereof or another antibody or fragment thereof that does not comprise the variable heavy chain region and/or variable light chain region of the SJ25C1 antibody, or portions thereof, such as one or more of the CDRs of the VH and/or VL regions. In some embodiments, the immunogen comprises the amino acid sequence set forth in SEQ ID NO:28 and SEQ ID NO:32, optionally separated by a linker, e.g. set forth in SEQ ID NO:33. In some embodiments, the antibody identified in (b) binds to the immunogen and does not bind to either an Fc domain or fragment thereof or a molecule comprising an scFv derived from the FMC63 antibody (such as an scFv comprising the amino acid sequence of SEQ ID NO: 34). In some embodiments, the antibody identified in (b) binds to a target molecule comprising the amino acid sequence of SEQ ID NO: 28 and does not bind to either a non-target molecule comprising the amino acid sequence of SEQ ID NO: 32 or a non-target molecule comprising the amino acid sequence of SEQ ID NO: 34.

[0271] In some embodiments, identifying the antibody comprises using hybridoma techniques to identify individual antibody clones produced by the subject and screening the antibody clones for binding to target and non-target molecules. In some embodiments, identifying the antibody comprises: (i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas; (ii) screening the hybridomas for production of antibodies that bind to the target molecule but not to the non-target molecule; and (iii) sequencing an antibody from a hybridoma producing an antibody that binds to the target molecule but not to the non-target molecule, thereby identifying the anti-idiotypic antibody. In some embodiments, screening a hybridoma comprises determining the binding affinity of the hybridoma antibody for the target molecule and the non-target molecule.

[0272] In an exemplary embodiment, the identification of anti-idiotypic antibodies recognizing the scFv portion of an exemplary anti-CD19 chimeric antigen receptor (CAR) containing an anti-CD19 scFv derived from SJ25C1 may be generated using an immunogen comprising the sequence set forth as follows:

EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQIYPGDG
DTNYNGKFKGQATLTADKSSSTAYMQLSGLTSEDSAVYFCARKTISSVVDIFYFDYW
QGQTTVTVSSGGGGSGGGGSGGGGSDIELTQSPKFMSTSVGDRVSVTCKASQNVGT
NVAWYQQKPGQSPKPLIYSATYRNSGVDPDRFTGSGSGTDFTLTITNVQSKDLADYFC
QQYNRYPYTSGGGTKLEIKR (SEQ ID NO: 28). In some embodiments, the immunogen
may further contain an Fc domain or fragment thereof, e.g. as set forth in SEQ ID NO:32.

[0273] In an exemplary embodiment, the identification of anti-idiotypic antibodies
recognizing the scFv portion of an exemplary anti-CD19 chimeric antigen receptor (CAR)
containing an anti-CD19 scFv derived from FMC63 may be generated using an immunogen
comprising the sequence set forth as follows:

DIQMTQTTSSLSASLGDRV TISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV
PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSGSGKPG
SGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
VIWGSETTYNSALKSRLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA
MDYWGQGTSTVTVSS (SEQ ID NO:34).

[0274] In some embodiments, the immunogen may further contain an Fc domain or
fragment thereof, e.g. as set forth in SEQ ID NO:32. In some embodiments, the identification of
anti-idiotypic antibodies recognizing the scFv portion of an exemplary anti-CD19 chimeric
antigen receptor (CAR) containing an anti-CD19 scFv derived from FMC63 may be generated
using an immunogen comprising the sequence set forth as follows:

DIQMTQTTSSLSASLGDRV TISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFS
GSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSGSGKPGSGEGSTKGEV
KLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALK
SRLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSTVTVSSPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 35).

III. CHIMERIC ANTIGEN RECEPTORS (CARs) AND GENETICALLY ENGINEERED CELLS

[0275] In some embodiments, the provided anti-idiotypic antibodies specifically bind to an antigen-binding portion of a chimeric antigen receptor (CAR), such as an anti-CD19 CAR containing an antigen-binding portion derived from antibody SJ25C1 or FMC63. In some embodiments, the provided anti-idiotypic antibodies bind to such CARs expressed on a cell, such as cells used in connection with adoptive cell therapy. In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering, and thereby express the recombinant or genetically engineered CAR products of such nucleic acids. In some embodiments, chimeric receptors when genetically engineered into immune cells can modulate T cell activity, and, in some cases, can modulate T cell differentiation or homeostasis, thereby resulting in genetically engineered cells with improved longevity, survival and/or persistence *in vivo*, such as for use in adoptive cell therapy methods. In some embodiments, the provided anti-idiotypic antibodies can be used in methods to modulate one or more of these activities, including to activate, stimulate and/or expand engineered cells expressing the target CAR.

[0276] In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering in accord with the provided methods, and thereby express recombinant or genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types. In particular embodiments, the nucleic acids contain a gene that encodes a CAR.

[0277] In some embodiments, the provided methods may be carried out simultaneously, sequentially or concurrently with one or more processing steps for manufacturing or preparing genetically engineered cells. The processing steps of the methods may include any one or more of a number of cell processing steps, alone or in combination. In particular embodiments, the processing steps include transduction or transfection of the cells with one or more nucleic acids,

e.g., a heterologous polynucleotide comprising a gene encoding a recombinant receptor. In certain embodiments, cells are transduced with viral vector particles containing a retroviral vector, such as one encoding a recombinant product for expression in the cells. In certain embodiments, the cells are transfected with one or more non-viral nucleic acids, e.g., an episomal plasmid or a transposon. The methods may further and/or alternatively include other processing steps, such as steps for the isolation, separation, selection, washing, suspension, dilution, concentration, and/or formulation of the cells. In some cases, the methods also can include an ex vivo step for cultivation, stimulation or expansion of cells (e.g., stimulation of the cells, for example, to induce their proliferation and/or activation), which, in some cases, can be carried out in accord with the provided methods. In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, and re-introducing them into the same subject, before or after cryopreservation.

[0278] In some embodiments, the method includes processing steps carried out in an order in which: cells, e.g., primary cells, are first isolated, such as selected or separated, from a biological sample; selected cells are incubated with viral vector particles for transduction; and transduced cells are formulated in a composition. In some cases, transduced cells are activated, expanded or propagated ex vivo, such as by stimulation in the presence of a stimulation reagent, such as in accord with the provided methods. In some embodiments, the method can include one or more processing steps from among washing, suspending, diluting and/or concentrating cells, which can occur prior to, during, or simultaneous with or subsequent to one or more of the isolation, such as separation or selection, transduction, stimulation, and/or formulation steps.

[0279] In particular embodiments, the cells to be transfected or transduced are not isolated, selected, or enriched prior to contact with the one or more nucleic acids. In some embodiments, the cells are not selected prior to contacting the cells with the one or more nucleic acids. In some embodiments, the cells to be transfected or transduced are not enriched prior to contacting the cells with the one or more nucleic acids.

[0280] In some embodiments, one or more of the cell processing steps in connection with preparing, processing and/or incubating cells in connection with the provided method, including in connection with preparing a composition containing genetically engineered cells, can be carried out in the internal cavity of a centrifugal chamber, such as a substantially rigid chamber

that is generally cylindrical in shape and rotatable around an axis of rotation, which can provide certain advantages compared to other available methods. In some embodiments, all processing steps are carried out in the same centrifugal chamber. In some embodiments, one or more processing steps are carried out in different centrifugal chambers, such as multiple centrifugal chambers of the same type. Such methods include any of those as described in International Publication Number WO2016/073602.

[0281] Exemplary centrifugal chambers include those produced and sold by Biosafe SA, including those for use with the Sepax® and Sepax® 2 system, including an A-200/F and A-200 centrifugal chambers and various kits for use with such systems. Exemplary chambers, systems, and processing instrumentation and cabinets are described, for example, in US Patent No. 6,123,655, US Patent No. 6,733,433 and Published U.S. Patent Application, Publication No.: US 2008/0171951, and published international patent application, publication no. WO 00/38762, the contents of each of which are incorporated herein by reference in their entirety. Depending on the particular process (e.g., dilution, wash, transduction, formulation), it is within the level of a skilled artisan to choose a particular kit that is appropriate for the process. Exemplary kits for use with such systems include, but are not limited to, single-use kits sold by BioSafe SA under product names CS-430.1, CS-490.1, CS-600.1 or CS-900.2.

[0282] In some embodiments, the system is included with and/or placed into association with other instrumentation, including instrumentation to operate, automate, control and/or monitor aspects of the various processing steps performed in the system. This instrumentation in some embodiments is contained within a cabinet. In some embodiments, the instrumentation includes a cabinet, which includes a housing containing control circuitry, a centrifuge, a cover, motors, pumps, sensors, displays, and a user interface. An exemplary device is described in US Patent No. 6,123,655, US Patent No. 6,733,433 and US 2008/0171951.

[0283] In some embodiments, the system comprises a series of containers, e.g., bags, tubing, stopcocks, clamps, connectors, and a centrifuge chamber. In some embodiments, the containers, such as bags, include one or more containers, such as bags, containing the cells to be transduced or transfected and the vector particles, e.g., viral vector particles or non-viral plasmids, in the same container or separate containers, such as the same bag or separate bags. In some embodiments, the system further includes one or more containers, such as bags, containing

medium, such as diluent and/or wash solution, which is pulled into the chamber and/or other components to dilute, resuspend, and/or wash components and/or compositions during the methods. The containers can be connected at one or more positions in the system, such as at a position corresponding to an input line, diluent line, wash line, waste line and/or output line.

[0284] In some embodiments, the system, such as a closed system, is sterile. In some embodiments, all connections of components of the system, such as between tubing line and a container via a connector, are made under sterile conditions. In some embodiments, connections are made under laminar flow. In some embodiments, connections are made using a sterile connection device that produces sterile connections, such as sterile welds, between a tubing and a container. In some embodiments, a sterile connection device effects connection under thermal condition high enough to maintain sterility, such as temperatures of at least 200 °C, such as at least 260 °C or 300 °C.

[0285] In some embodiments, the system may be disposable, such as a single-use kit. In some embodiments, a single-use kit can be utilized in a plurality of cycles of a process or processes, such as at least 2, 3, 4, 5 or more times, for example, in processes that occur in a continuous or a semi-continuous manner. In some embodiments, the system, such as a single-use kit, is employed for processing of cells from a single patient. In aspects of the methods, the processes need not be performed in the same closed system, such as in the same centrifugal chamber, but can be performed under a different closed system, such as in a different centrifugal chamber; in some embodiments, such different centrifugal chambers are at the respective points in the methods placed in association with the same system, such as placed in association with the same centrifuge. In some embodiments, all processing steps are performed in a closed system, in which all or a subset of each one or more processing step is performed in the same or a different centrifugal chamber.

A. Target Chimeric Antigen Receptors (CARs)

[0286] In some embodiments, the provided anti-idiotypic antibodies specifically bind the extracellular domain of a target CAR that contains an antigen binding domain of an antibody or antibody fragment that provides specificity for a desired antigen (e.g., tumor antigen) and which is operably linked or connected to an intracellular signaling domain. In some embodiments, the

antigen binding domain includes the antibody SJ25C1 or an antibody fragment of portion derived from SJ25C1. In some embodiments, the antigen binding domain includes the antibody FMC63 or an antibody fragment of portion derived from FMC63. In some embodiments, the intracellular signaling domain is an activating intracellular domain portion, such as a T cell activating domain, providing a primary activation signal. In some embodiments, the intracellular signaling domain contains or additionally contains a costimulatory signaling domain to facilitate effector functions.

[0287] In some embodiments, engineered cells, such as T cells, are provided that express a CAR with specificity for a particular antigen (or marker or ligand), such as an antigen expressed on the surface of a particular cell type. In some embodiments, the antigen is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen is selectively expressed or overexpressed on cells of the disease or condition, e.g., the tumor or pathogenic cells, as compared to normal or non-targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0288] In particular embodiments, the recombinant receptor, such as a chimeric receptor, contains an intracellular signaling region, which includes a cytoplasmic signaling domain (also interchangeably called an intracellular signaling domain), such as a cytoplasmic (intracellular) region capable of inducing a primary activation signal in a T cell, for example, a cytoplasmic signaling domain of a T cell receptor (TCR) component (e.g. a cytoplasmic signaling domain of a zeta chain of a CD3-zeta (CD3 ζ) chain or a functional variant or signaling portion thereof) that comprises an immunoreceptor tyrosine-based activation motif (ITAM).

[0289] In some embodiments, the chimeric receptor further contains an extracellular ligand-binding domain that specifically binds to a ligand (e.g. antigen). In some embodiments, the chimeric receptor is a CAR that contains an extracellular antigen-recognition domain that specifically binds to an antigen. In some embodiments, the ligand, such as an antigen, is a protein expressed on the surface of cells. In some embodiments, the CAR is a TCR-like CAR and the antigen is a processed peptide antigen, such as a peptide antigen of an intracellular protein, which, like a TCR, is recognized on the cell surface in the context of a major histocompatibility complex (MHC) molecule.

[0290] Exemplary antigen receptors, including CARs, and methods for engineering and introducing such receptors into cells, include those described, for example, in international patent application publication numbers WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013/166321, WO2013/071154, WO2013/123061 U.S. patent application publication numbers US2002131960, US2013287748, US20130149337, U.S. Patent Nos.: 6,451,995, 7,446,190, 8,252,592, , 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application number EP2537416, and/or those described by Sadelain et al., *Cancer Discov.* 2013 April; 3(4): 388–398; Davila et al. (2013) *PLoS ONE* 8(4): e61338; Turtle et al., *Curr. Opin. Immunol.*, 2012 October; 24(5): 633-39; Wu et al., *Cancer*, 2012 March 18(2): 160-75. In some aspects, the antigen receptors include a CAR as described in U.S. Patent No.: 7,446,190, and those described in International Patent Application Publication No.: WO/2014055668 A1. Examples of the CARs include CARs as disclosed in any of the aforementioned publications, such as WO2014031687, U.S. Patent No. 8,339,645, U.S. Patent No. 7,446,179, US 2013/0149337, U.S. Patent No.: 7,446,190, U.S. Patent No.: 8,389,282, Kochenderfer et al., 2013, *Nature Reviews Clinical Oncology*, 10, 267-276 (2013); Wang et al. (2012) *J. Immunother.* 35(9): 689-701; and Brentjens et al., *Sci Transl Med.* 2013 5(177). See also WO2014031687, U.S. Patent No. 8,339,645, U.S. Patent No. 7,446,179, US 2013/0149337, U.S. Patent No.: 7,446,190, and U.S. Patent No.: 8,389,282.

[0291] In some embodiments, the CAR is constructed with a specificity for a particular antigen (or marker or ligand), such as an antigen expressed in a particular cell type to be targeted by adoptive therapy, *e.g.*, a cancer marker, and/or an antigen intended to induce a dampening response, such as an antigen expressed on a normal or non-diseased cell type. Thus, the CAR typically includes in its extracellular portion one or more antigen binding molecules, such as one or more antigen-binding fragment, domain, or portion, or one or more antibody variable domains, and/or antibody molecules. In some embodiments, the CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (VH) and variable light (VL) chains of a monoclonal antibody (mAb).

[0292] In some embodiments, the antibody or antigen-binding portion thereof is expressed on cells as part of a CAR. Generally, a CAR containing an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against peptide-MHC complexes also may be referred to as a TCR-like CAR. In some embodiments, the extracellular antigen binding domain specific for an MHC-peptide complex of a TCR-like CAR is linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). In some embodiments, such molecules can typically mimic or approximate a signal through a natural antigen receptor, such as a TCR, and, optionally, a signal through such a receptor in combination with a costimulatory receptor.

[0293] In some embodiments, the recombinant receptor, such as a chimeric receptor (e.g. CAR), includes a ligand-binding domain that binds, such as specifically binds, to an antigen (or a ligand). Among the antigens targeted by the chimeric receptors are those expressed in the context of a disease, condition, or cell type to be targeted via the adoptive cell therapy. Among the diseases and conditions are proliferative, neoplastic, and malignant diseases and disorders, including cancers and tumors, including hematologic cancers, cancers of the immune system, such as lymphomas, leukemias, and/or myelomas, such as B, T, and myeloid leukemias, lymphomas, and multiple myelomas.

[0294] In some embodiments, the antigen (or a ligand) is a polypeptide. In some embodiments, it is a carbohydrate or other molecule. In some embodiments, the antigen (or a ligand) is selectively expressed or overexpressed on cells of the disease or condition, *e.g.*, the tumor or pathogenic cells, as compared to normal or non-targeted cells or tissues. In other embodiments, the antigen is expressed on normal cells and/or is expressed on the engineered cells.

[0295] In some embodiments, the CAR contains an antibody or an antigen-binding fragment (*e.g.* scFv) that specifically recognizes an antigen, such as an intact antigen, expressed on the surface of a cell.

[0296] In some embodiments, the antigen (or a ligand) is a tumor antigen or cancer marker. In some embodiments, the antigen (or a ligand) is $\alpha\beta6$ integrin ($\alpha\beta6$ integrin), B cell maturation antigen (BCMA), B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and

LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, epidermal growth factor protein (EGFR), truncated epidermal growth factor protein (tEGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrine receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, fetal acetylcholine receptor, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), Her2/neu (receptor tyrosine kinase erbB2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-AI), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific antigen, an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by and/or characteristic of or specific for HIV, HCV, HBV, HPV, and/or other pathogens and/or oncogenic versions thereof. Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell markers. In some embodiments, the antigen targeted by the receptor is CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30.

[0297] In some embodiments, the antigen is a pathogen-specific antigen. In some embodiments, the antigen is a viral antigen (such as a viral antigen from HIV, HCV, HBV, etc.), bacterial antigens, and/or parasitic antigens.

[0298] Antigens targeted by the receptors in some embodiments include antigens associated with a B cell malignancy, such as any of a number of known B cell marker. In some embodiments, the antigen targeted by the receptor is CD20, CD19, CD22, ROR1, CD45, CD21, CD5, CD33, Igkappa, Iglambda, CD79a, CD79b or CD30. In some embodiments, the antigen is CD19 and is specifically bound by an anti-CD19 antibody, such as SJ25C1 or an antigen-binding fragment derived from SJ25C1 or FMC63 or an antigen-binding fragment derived from FMC63.

[0299] In some embodiments, the antigen-binding proteins, antibodies and antigen binding fragments thereof specifically recognize an antigen of a full-length antibody. In some embodiments, the heavy and light chains of an antibody can be full-length or can be an antigen-binding portion (a Fab, F(ab')₂, Fv or a single chain Fv fragment (scFv)). In other embodiments, the antibody heavy chain constant region is chosen from, e.g., IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE, particularly chosen from, e.g., IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (e.g., human IgG1). In another embodiment, the antibody light chain constant region is chosen from, e.g., kappa or lambda, particularly kappa.

[0300] Among the provided antibodies are antibody fragments. An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; variable heavy chain (VH) regions, single-chain antibody molecules such as scFvs and single-domain VH single antibodies; and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0301] The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and

three CDRs. (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0302] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody. In some embodiments, the CAR comprises an antibody heavy chain domain that specifically binds the antigen, such as a cancer marker or cell surface antigen of a cell or disease to be targeted, such as a tumor cell or a cancer cell, such as any of the target antigens described herein or known in the art.

[0303] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, e.g., peptide linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some embodiments, the antibody fragments are scFvs.

[0304] A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0305] The chimeric receptors, such as CARs, generally include an extracellular antigen binding domain, such as a portion of an antibody molecule (e.g. SJ25C1 or FMC63), generally a variable heavy (VH) chain region and/or variable light (VL) chain region of the antibody, e.g., an scFv antibody fragment.

[0306] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing an antibody or antibody fragment. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment and an intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv. In some embodiments, the scFv is derived from SJ25C1 and comprises the sequence of amino acids set forth in SEQ ID NO: 28. In some embodiments, the scFv is derived from FMC63 and comprises the sequence of amino acids set forth in SEQ ID NO: 34.

[0307] In some embodiments, the recombinant receptor such as the CAR, such as the antibody portion thereof, further includes a spacer, which may be or include at least a portion of an immunoglobulin constant region or variant or modified version thereof, such as a hinge region, e.g., an IgG4 hinge region, and/or a CH1/CL and/or Fc region. In some embodiments, the constant region or portion is of a human IgG, such as IgG4 or IgG1. In some aspects, the portion of the constant region serves as a spacer region between the antigen-recognition component, e.g., scFv, and transmembrane domain. The spacer can be of a length that provides for increased responsiveness of the cell following antigen binding, as compared to in the absence of the spacer. In some examples, the spacer is at or about 12 amino acids in length or is no more than 12 amino acids in length. Exemplary spacers include those having at least about 10 to 229 amino acids, about 10 to 200 amino acids, about 10 to 175 amino acids, about 10 to 150 amino acids, about 10 to 125 amino acids, about 10 to 100 amino acids, about 10 to 75 amino acids, about 10 to 50 amino acids, about 10 to 40 amino acids, about 10 to 30 amino acids, about 10 to 20 amino acids, or about 10 to 15 amino acids, and including any integer between the endpoints of any of the listed ranges. In some embodiments, a spacer region has about 12 amino acids or less, about 119 amino acids or less, or about 229 amino acids or less. Exemplary spacers include IgG4 hinge alone, IgG4 hinge linked to CH2 and CH3 domains, or IgG4 hinge linked to the CH3 domain. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 150, and is encoded by the sequence set forth in SEQ ID NO: 151. In some embodiments, the

spacer has the sequence set forth in SEQ ID NO: 152. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 153. Exemplary spacers include, but are not limited to, those described in Hudecek *et al.* (2013) *Clin. Cancer Res.*, 19: 3153, international patent application publication number WO2014031687, U.S. Patent No. 8,822,647 or published app. No. US2014/0271635.

[0308] In some embodiments, the constant region or portion is of IgD. In some embodiments, the spacer has the sequence set forth in SEQ ID NO: 154. In some embodiments, the spacer has a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1, 3, 4 and 5.

[0309] In some embodiments, the antigen receptor comprises an intracellular domain linked directly or indirectly to the extracellular domain. In some embodiments, the chimeric antigen receptor includes a transmembrane domain linking the extracellular domain and the intracellular signaling domain. In some embodiments, the transmembrane domain is fused to the extracellular domain. In some embodiments, the intracellular signaling domain comprises an ITAM. For example, in some aspects, the antigen recognition domain (e.g. extracellular domain) generally is linked to one or more intracellular signaling components, such as signaling components that mimic activation through an antigen receptor complex, such as a TCR complex, in the case of a CAR, and/or signal via another cell surface receptor. In some embodiments, the chimeric receptor comprises a transmembrane domain linked or fused between the extracellular domain (e.g. scFv) and intracellular signaling domain. Thus, in some embodiments, the antigen-binding component (e.g., antibody) is linked to one or more transmembrane and intracellular signaling domains.

[0310] In one embodiment, a transmembrane domain that naturally is associated with one of the domains in the receptor, e.g., CAR, is used. In some instances, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0311] The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane regions include those derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD 16, CD22, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD 154. Alternatively the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. In some embodiments, the linkage is by linkers, spacers, and/or transmembrane domain(s). In some aspects, the transmembrane domain contains a transmembrane portion of CD28.

[0312] In some embodiments, the extracellular domain and transmembrane domain can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein. In some embodiments, the receptor contains extracellular portion of the molecule from which the transmembrane domain is derived, such as a CD28 extracellular portion.

[0313] Among the intracellular signaling domains are those that mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, a short oligo- or polypeptide linker, for example, a linker of between 2 and 10 amino acids in length, such as one containing glycines and serines, e.g., glycine-serine doublet, is present and forms a linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR.

[0314] T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the CAR includes one or both of such signaling components.

[0315] The receptor, e.g., the CAR, generally includes at least one intracellular signaling component or components. In some aspects, the CAR includes a primary cytoplasmic signaling sequence that regulates primary activation of the TCR complex. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences include those derived from CD3 zeta chain, FcR gamma, CD3 gamma, CD3 delta and CD3 epsilon. In some embodiments, cytoplasmic signaling molecule(s) in the CAR contain(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3 zeta.

[0316] In some embodiments, the receptor includes an intracellular component of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, e.g., CD3 zeta chain. Thus, in some aspects, the antigen-binding portion is linked to one or more cell signaling modules. In some embodiments, cell signaling modules include CD3 transmembrane domain, CD3 intracellular signaling domains, and/or other CD transmembrane domains. In some embodiments, the receptor, e.g., CAR, further includes a portion of one or more additional molecules such as Fc receptor γ , CD8, CD4, CD25, or CD16. For example, in some aspects, the CAR or other chimeric receptor includes a chimeric molecule between CD3-zeta (CD3- ζ) or Fc receptor γ and CD8, CD4, CD25 or CD16.

[0317] In some embodiments, upon ligation of the CAR or other chimeric receptor, the cytoplasmic domain or intracellular signaling domain of the receptor activates at least one of the normal effector functions or responses of the immune cell, e.g., T cell engineered to express the CAR. For example, in some contexts, the CAR induces a function of a T cell such as cytolytic activity or T-helper activity, such as secretion of cytokines or other factors. In some embodiments, a truncated portion of an intracellular signaling domain of an antigen receptor component or costimulatory molecule is used in place of an intact immunostimulatory chain, for example, if it transduces the effector function signal. In some embodiments, the intracellular signaling domain or domains include the cytoplasmic sequences of the T cell receptor (TCR), and in some aspects also those of co-receptors that in the natural context act in concert with such receptors to initiate signal transduction following antigen receptor engagement, and/or any

derivative or variant of such molecules, and/or any synthetic sequence that has the same functional capability.

[0318] In the context of a natural TCR, full activation generally requires not only signaling through the TCR, but also a costimulatory signal. Thus, in some embodiments, to promote full activation, a component for generating secondary or co-stimulatory signal is also included in the CAR. In other embodiments, the CAR does not include a component for generating a costimulatory signal. In some aspects, an additional CAR is expressed in the same cell and provides the component for generating the secondary or costimulatory signal.

[0319] T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the CAR includes one or both of such signaling components.

[0320] In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule. In some embodiments, the CAR includes a signaling domain and/or transmembrane portion of a costimulatory receptor, such as CD28, 4-1BB, OX40, DAP10, and ICOS. In some aspects, the same CAR includes both the activating and costimulatory components. In some embodiments, the chimeric antigen receptor contains an intracellular domain derived from a T cell costimulatory molecule or a functional variant thereof, such as between the transmembrane domain and intracellular signaling domain. In some aspects, the T cell costimulatory molecule is CD28 or 41BB.

[0321] In some embodiments, the activating domain is included within one CAR, whereas the costimulatory component is provided by another CAR recognizing another antigen. In some embodiments, the CARs include activating or stimulatory CARs, costimulatory CARs, both expressed on the same cell (see WO2014/055668). In some aspects, the cells include one or more stimulatory or activating CAR and/or a costimulatory CAR. In some embodiments, the cells further include inhibitory CARs (iCARs, see Fedorov et al., *Sci. Transl. Medicine*, 5(215) (December, 2013), such as a CAR recognizing an antigen other than the one associated with and/or specific for the disease or condition whereby an activating signal delivered through the

disease-targeting CAR is diminished or inhibited by binding of the inhibitory CAR to its ligand, e.g., to reduce off-target effects.

[0322] In certain embodiments, the intracellular signaling domain comprises a CD28 transmembrane and signaling domain linked to a CD3 (e.g., CD3-zeta) intracellular domain. In some embodiments, the intracellular signaling domain comprises a chimeric CD28 and CD137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

[0323] In some embodiments, the CAR encompasses one or more, e.g., two or more, costimulatory domains and an activation domain, e.g., primary activation domain, in the cytoplasmic portion. Exemplary CARs include intracellular components of CD3-zeta, CD28, and 4-1BB.

[0324] In some embodiments, the antigen receptor further includes a marker and/or cells expressing the CAR or other antigen receptor further includes a surrogate marker, such as a cell surface marker, which may be used to confirm transduction or engineering of the cell to express the receptor. In some aspects, the marker includes all or part (e.g., truncated form) of CD34, a NGFR, or epidermal growth factor receptor, such as truncated version of such a cell surface receptor (e.g., tEGFR). In some embodiments, the nucleic acid encoding the marker is operably linked to a polynucleotide encoding for a linker sequence, such as a cleavable linker sequence, e.g., T2A. For example, a marker, and optionally a linker sequence, can be any as disclosed in published patent application No. WO2014031687. For example, the marker can be a truncated EGFR (tEGFR) that is, optionally, linked to a linker sequence, such as a T2A cleavable linker sequence. In embodiments, the tEGFR contains an amino acid sequence set forth in SEQ ID NO: 155 or 156. In some embodiments, the tEGFR contains an amino acid sequence with or with about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99% sequence identity to the sequences set forth in SEQ ID NO: 155 or 156.

[0325] In some embodiments, the marker is a molecule, e.g., cell surface protein, not naturally found on T cells or not naturally found on the surface of T cells, or a portion thereof. In some embodiments, the molecule is a non-self molecule, e.g., non-self protein, i.e., one that is not recognized as “self” by the immune system of the host into which the cells will be adoptively transferred.

[0326] In some embodiments, the marker serves no therapeutic function and/or produces no effect other than to be used as a marker for genetic engineering, e.g., for selecting cells successfully engineered. In other embodiments, the marker may be a therapeutic molecule or molecule otherwise exerting some desired effect, such as a ligand for a cell to be encountered in vivo, such as a costimulatory or immune checkpoint molecule to enhance and/or dampen responses of the cells upon adoptive transfer and encounter with ligand.

[0327] In some cases, CARs are referred to as first, second, and/or third generation CARs. In some aspects, a first generation CAR is one that solely provides a CD3-chain induced signal upon antigen binding; in some aspects, a second-generation CARs is one that provides such a signal and costimulatory signal, such as one including an intracellular signaling domain from a costimulatory receptor such as CD28 or CD137; in some aspects, a third generation CAR is one that includes multiple costimulatory domains of different costimulatory receptors.

[0328] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein and an intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv or a single-domain V_H antibody and the intracellular domain contains an ITAM. In some aspects, the intracellular signaling domain includes a signaling domain of a zeta chain of a CD3-zeta (CD3 ζ) chain. In some embodiments, the chimeric antigen receptor includes a transmembrane domain disposed between the extracellular domain and the intracellular signaling region.

[0329] In some aspects, the transmembrane domain contains a transmembrane portion of CD28. The extracellular domain and transmembrane can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein. In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule, such as between the transmembrane domain and intracellular signaling domain. In some aspects, the T cell costimulatory molecule is CD28 or 4-1BB.

[0330] For example, in some embodiments, the CAR contains an antibody, e.g., an antibody fragment, a transmembrane domain that is or contains a transmembrane portion of CD28 or a functional variant thereof, and an intracellular signaling domain containing a signaling portion of CD28 or functional variant thereof and a signaling portion of CD3 zeta or functional variant thereof. In some embodiments, the CAR contains an antibody, e.g., antibody fragment, a transmembrane domain that is or contains a transmembrane portion of CD28 or a functional variant thereof, and an intracellular signaling domain containing a signaling portion of a 4-1BB or functional variant thereof and a signaling portion of CD3 zeta or functional variant thereof. In some such embodiments, the receptor further includes a spacer containing a portion of an Ig molecule, such as a human Ig molecule, such as an Ig hinge, e.g. an IgG4 hinge, such as a hinge-only spacer.

[0331] In some embodiments, the transmembrane domain of the receptor, e.g., the CAR is a transmembrane domain of human CD28 or variant thereof, e.g., a 27-amino acid transmembrane domain of a human CD28 (Accession No.: P10747.1), or is a transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 157 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:157; in some embodiments, the transmembrane-domain containing portion of the recombinant receptor comprises the sequence of amino acids set forth in SEQ ID NO: 158 or a sequence of amino acids having at least at or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto.

[0332] In some embodiments, the chimeric antigen receptor contains an intracellular domain of a T cell costimulatory molecule. In some aspects, the T cell costimulatory molecule is CD28 or 4-1BB.

[0333] In some embodiments, the intracellular signaling region comprises an intracellular costimulatory signaling domain of human CD28 or functional variant or portion thereof, such as a 41 amino acid domain thereof and/or such a domain with an LL to GG substitution at positions 186-187 of a native CD28 protein. In some embodiments, the intracellular signaling domain can comprise the sequence of amino acids set forth in SEQ ID NO: 159 or 160 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 159 or 160. In some embodiments, the intracellular region comprises an intracellular costimulatory signaling domain of 4-1BB or functional variant or portion thereof, such as a 42-amino acid cytoplasmic domain of a human 4-1BB (Accession No. Q07011.1) or functional variant or portion thereof, such as the sequence of amino acids set forth in SEQ ID NO: 161 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 161.

[0334] In some embodiments, the intracellular signaling region comprises a human CD3 chain, optionally a CD3 zeta stimulatory signaling domain or functional variant thereof, such as an 112 AA cytoplasmic domain of isoform 3 of human CD3 ζ (Accession No.: P20963.2) or a CD3 zeta signaling domain as described in U.S. Patent No.: 7,446,190 or U.S. Patent No. 8,911,993. In some embodiments, the intracellular signaling region comprises the sequence of amino acids set forth in SEQ ID NO: 162, 163 or 164 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 162, 163 or 164.

[0335] In some aspects, the spacer contains only a hinge region of an IgG, such as only a hinge of IgG4 or IgG1, such as the hinge only spacer set forth in SEQ ID NO: 150. In other embodiments, the spacer is an Ig hinge, e.g., and IgG4 hinge, linked to a C_H2 and/or C_H3 domains. In some embodiments, the spacer is an Ig hinge, e.g., an IgG4 hinge, linked to C_H2 and C_H3 domains, such as set forth in SEQ ID NO: 152. In some embodiments, the spacer is an Ig hinge, e.g., an IgG4 hinge, linked to a C_H3 domain only, such as set forth in SEQ ID NO: 153. In some embodiments, the spacer is or comprises a glycine-serine rich sequence or other flexible linker such as known flexible linkers.

[0336] For example, in some embodiments, the CAR includes an antibody such as an antibody fragment, including scFvs, a spacer, such as a spacer containing a portion of an immunoglobulin molecule, such as a hinge region and/or one or more constant regions of a heavy chain molecule, such as an Ig-hinge containing spacer, a transmembrane domain containing all or a portion of a CD28-derived transmembrane domain, a CD28-derived intracellular signaling domain, and a CD3 zeta signaling domain. In some embodiments, the CAR includes an antibody or fragment, such as scFv, a spacer such as any of the Ig-hinge

containing spacers, a CD28-derived transmembrane domain, a 4-1BB-derived intracellular signaling domain, and a CD3 zeta-derived signaling domain.

[0337] In some embodiments, nucleic acid molecules encoding such CAR constructs further includes a sequence encoding a T2A ribosomal skip element and/or a tEGFR sequence, e.g., downstream of the sequence encoding the CAR. In some embodiments, T cells expressing an antigen receptor (e.g. CAR) can also be generated to express a truncated EGFR (EGFRt) as a non-immunogenic selection epitope (e.g. by introduction of a construct encoding the CAR and EGFRt separated by a T2A ribosome switch to express two proteins from the same construct), which then can be used as a marker to detect such cells (see e.g. U.S. Patent No. 8,802,374). In some embodiments, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (e.g. encoding the molecule involved in modulating a metabolic pathway and encoding the recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (e.g., 2A sequences) or a protease recognition site (e.g., furin). The ORF thus encodes a single polypeptide, which, either during (in the case of 2A) or after translation, is processed into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (see, for example, de Felipe. *Genetic Vaccines and Ther.* 2:13 (2004) and deFelipe *et al. Traffic* 5:616-626 (2004)). Many 2A elements are known in the art. Examples of 2A sequences that can be used in the methods and nucleic acids disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A, e.g., SEQ ID NO: 131), equine rhinitis A virus (E2A, e.g., SEQ ID NO: 130), Thosa asigna virus (T2A, e.g., SEQ ID NO: 126 or 127), and porcine teschovirus-1 (P2A, e.g., SEQ ID NO: 128 or 129) as described in U.S. Patent Publication No. 20070116690.

[0338] The recombinant receptors, such as CARs, expressed by the cells administered to the subject generally recognize or specifically bind to a molecule that is expressed in, associated with, and/or specific for the disease or condition or cells thereof being treated. Upon specific binding to the molecule, e.g., antigen, the receptor generally delivers an immunostimulatory signal, such as an ITAM-transduced signal, into the cell, thereby promoting an immune response targeted to the disease or condition. For example, in some embodiments, the cells express a

CAR that specifically binds to an antigen expressed by a cell or tissue of the disease or condition or associated with the disease or condition. The receptor may be another receptor such as an immunoinhibitory or costimulatory signal-promoting receptor, such as a CCR or iCAR or non-signaling receptor, e.g., for use in depletion or elimination of cells using the antibodies.

B. Genetically Engineered Cells and Methods of Producing Cells

[0339] Among the cells expressing the chimeric antigen receptors are engineered cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into a composition containing the cells, such as by retroviral transduction, transfection, or transformation. Various methods for the introduction of genetically engineered components, e.g., recombinant receptors, e.g., CARs, are well known and may be used. Exemplary methods include those for transfer of nucleic acids encoding the receptors, including via viral, e.g., retroviral or lentiviral, transduction, transposons, and electroporation.

1. Vectors and Methods for Genetic Engineering

[0340] Also provided are one or more polynucleotides (e.g., nucleic acid molecules) encoding chimeric antigen receptors (CARs), vectors for genetically engineering cells to express such CARs and methods for producing the engineered cells. In some embodiments, the vector contains the nucleic acid encoding the CAR. In some cases, the vector is a viral vector, such as a retroviral vector, e.g., a lentiviral vector or a gammaretroviral vector.

[0341] In some embodiments, recombinant nucleic acids are transferred into cells using recombinant infectious virus particles, such as, e.g., vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant nucleic acids are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors (see, e.g., Koste et al. (2014) *Gene Therapy* 2014 Apr 3. doi: 10.1038/gt.2014.25; Carlens et al. (2000) *Exp Hematol* 28(10): 1137-46; Alonso-Camino et al. (2013) *Mol Ther Nucl Acids* 2, e93; Park et al., *Trends Biotechnol.* 2011 November 29(11): 550–557.

[0342] In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), e.g., a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine

stem cell virus (MSCV), spleen focus forming virus (SFFV), or adeno-associated virus (AAV). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In one embodiment, the gene to be expressed replaces the retroviral gag, pol and/or env sequences. A number of illustrative retroviral systems have been described (e.g., U.S. Pat. Nos. 5,219,740; 6,207,453; 5,219,740; Miller and Rosman (1989) *BioTechniques* 7: 980-990; Miller, A. D. (1990) *Human Gene Therapy* 1: 5-14; Scarpa et al. (1991) *Virology* 180: 849-852; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90: 8033-8037; and Boris-Lawrie and Temin (1993) *Cur. Opin. Genet. Develop.* 3: 102-109.

[0343] Methods of lentiviral transduction are known. Exemplary methods are described in, e.g., Wang et al. (2012) *J. Immunother.* 35(9): 689-701; Cooper et al. (2003) *Blood*. 101: 1637-1644; Verhoeven et al. (2009) *Methods Mol Biol.* 506: 97-114; and Cavalieri et al. (2003) *Blood*. 102(2): 497-505.

[0344] In some embodiments, recombinant nucleic acids are transferred into T cells via electroporation (see, e.g., Chicaybam et al, (2013) *PLoS ONE* 8(3): e60298 and Van Tedeloo et al. (2000) *Gene Therapy* 7(16): 1431-1437). In some embodiments, recombinant nucleic acids are transferred into T cells via transposition (see, e.g., Manuri et al. (2010) *Hum Gene Ther* 21(4): 427-437; Sharma et al. (2013) *Molec Ther Nucl Acids* 2, e74; and Huang et al. (2009) *Methods Mol Biol* 506: 115-126). Other methods of introducing and expressing genetic material in immune cells include calcium phosphate transfection (e.g., as described in *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.), protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.*, 7: 2031-2034 (1987)).

[0345] Other approaches and vectors for transfer of the nucleic acids encoding the recombinant products are those described, e.g., in international patent application, Publication No.: WO2014055668, and U.S. Patent No. 7,446,190.

[0346] In some embodiments, the cells, e.g., T cells, may be transfected either during or after expansion, e.g. with a T cell receptor (TCR) or a chimeric antigen receptor (CAR). This transfection for the introduction of the gene of the desired receptor can be carried out with any suitable retroviral vector, for example. The genetically modified cell population can then be liberated from the initial stimulus (the anti-CD3/anti-CD28 stimulus, for example) and subsequently be stimulated with a second type of stimulus e.g. via a de novo introduced receptor). This second type of stimulus may include an antigenic stimulus in form of a peptide/MHC molecule, the cognate (cross-linking) ligand of the genetically introduced receptor (e.g. natural ligand of a CAR) or any ligand (such as an antibody) that directly binds within the framework of the new receptor (e.g. by recognizing constant regions within the receptor). See, for example, Cheadle et al, "Chimeric antigen receptors for T-cell based therapy" *Methods Mol Biol.* 2012; 907: 645-66 or Barrett et al., *Chimeric Antigen Receptor Therapy for Cancer Annual Review of Medicine* Vol. 65: 333-347 (2014). In some embodiments, the cells are stimulated with a provided anti-idiotypic antibody in accord with the provided methods.

[0347] In some cases, a vector may be used that does not require that the cells, e.g., T cells, are activated. In some such instances, the cells may be selected and/or transduced prior to activation. Thus, the cells may be engineered prior to, or subsequent to culturing of the cells, and in some cases at the same time as or during at least a portion of the culturing.

[0348] Among additional nucleic acids, e.g., genes for introduction are those to improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess *in vivo* survival or localization; genes to improve safety, for example, by making the cell susceptible to negative selection *in vivo* as described by Lupton S. D. et al., *Mol. and Cell Biol.*, 11: 6 (1991); and Riddell et al., *Human Gene Therapy* 3: 319-338 (1992); see also the publications of PCT/US91/08442 and PCT/US94/05601 by Lupton et al. describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker. See, e.g., Riddell et al., U.S. Patent No. 6,040,177, at columns 14-17.

2. Cells and Preparation of Cells for Genetic Engineering

[0349] Provided herein are cells, including engineered cells that contain a chimeric antigen receptor (CAR). Also provided are population of such cells and compositions containing such cells. Among the compositions are input compositions containing cells in which one or more cells is known or likely or will express a recombinant receptor capable of being recognized or bound by a binding molecule present on one or more particles to which the cells are incubated or contacted. Also among the compositions are compositions produced by the provided methods, including output compositions in which is contained stimulated or expanded cells, including compositions enriched for cells containing a recombinant receptor bound or recognized by the binding molecule of the particle, such as in which cells expressing the recombinant receptor, e.g. chimeric receptor, make up at least 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or more percent of the total cells in the composition or cells of a certain type such as T cells or CD8+ or CD4+ cells. Thus, provided are genetically engineered cells expressing the recombinant receptors e.g., CARs.

[0350] Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are methods for engineering, producing or generating such cells, therapeutic methods for administering the cells and compositions to subjects, e.g., patients, and methods for detecting, selecting, isolating or separating such cells.

[0351] In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0352] The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, e.g., myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK

cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs).

[0353] The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, and re-introducing them into the same subject, before or after cryopreservation.

[0354] Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naïve T (T_N) cells, effector T cells (T_{EFF}), memory T cells and sub-types thereof, such as stem cell memory T (T_{SCM}), central memory T (T_{CM}), effector memory T (T_{EM}), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0355] In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, e.g., myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0356] In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism

or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0357] In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the nucleic acid encoding the transgenic receptor such as the CAR, may be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

[0358] Accordingly, the cells in some embodiments are primary cells, e.g., primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g. transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0359] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, e.g., adoptive cell therapy, samples from autologous and allogeneic sources.

[0360] In some embodiments, the cells are derived from cell lines, e.g., T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, and pig.

[0361] In some embodiments, isolation of the cells includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

[0362] In some examples, cells from the circulating blood of a subject are obtained, e.g., by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets. In some embodiments, prior to the selection and/or enrichment of cells, the sample or the cells in the sample can be rested or held prior to further processing steps. In some embodiments, the sample is maintained at or held at a temperature of from or from about 2° C to 8° C for up to 48 hours, such as for up to 12 hours, 24 hours or 36 hours. In certain embodiments, the cells are not selected and/or enriched prior to contacting the cells with the one or more nucleic acids. In some embodiments, the sample or the cells can be rested or held prior to contacting or incubating the cells with one or more nucleic acids. In certain embodiments, the sample is maintained at or held at a temperature of from or from about 2° C to 8° C for up to 48 hours, such as for up to 12 hours, 24 hours or 36 hours prior to contacting or incubating the cells with one or more nucleic acids.

[0363] In some embodiments, the blood cells collected from the subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer's instructions. In some aspects, a washing step is accomplished by

tangential flow filtration (TFF) according to the manufacturer's instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, $\text{Ca}^{++}/\text{Mg}^{++}$ free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0364] In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

[0365] In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, e.g., surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers may be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[0366] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

[0367] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular

type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells.

[0368] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[0369] For example, in some aspects, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, e.g., CD28⁺, CD62L⁺, CCR7⁺, CD27⁺, CD127⁺, CD4⁺, CD8⁺, CD45RA⁺, and/or CD45RO⁺ T cells, are isolated by positive or negative selection techniques.

[0370] For example, CD3⁺, CD28⁺ T cells can be positively selected using CD3/CD28 conjugated magnetic beads (e.g., DYNABEADS® M-450 CD3/CD28 T Cell Expander). In particular embodiments, cells are contacted with anti-CD3/anti-CD28 conjugated magnetic beads (e.g., DYNABEADS® M-450 CD3/CD28 T Cell Expander) to expand CD3⁺, CD28⁺ T cells prior to contacting the cells with the one or more nucleic acids. In certain embodiments, the cells are not contacted with anti-CD3/anti-CD28 conjugated magnetic beads prior to contacting the cells with the one or more nucleic acids.

[0371] In some embodiments, isolation is carried out by enrichment for a particular cell population by positive selection, or depletion of a particular cell population, by negative selection. In some embodiments, positive or negative selection is accomplished by incubating cells with one or more antibodies or other binding agent that specifically bind to one or more surface markers expressed or expressed (marker⁺) at a relatively higher level (marker^{high}) on the positively or negatively selected cells, respectively.

[0372] In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD14. In some aspects, a CD4⁺ or CD8⁺ selection step is used to separate CD4⁺ helper and CD8⁺ cytotoxic T cells. Such CD4⁺ and CD8⁺ populations can be further sorted into

sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations.

[0373] In some embodiments, CD8⁺ cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (T_{CM}) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such sub-populations. *See* Terakura et al. (2012) *Blood*.1: 72–82; Wang et al. (2012) *J Immunother.* 35(9): 689-701. In some embodiments, combining T_{CM}-enriched CD8⁺ T cells and CD4⁺ T cells further enhances efficacy.

[0374] In embodiments, memory T cells are present in both CD62L⁺ and CD62L⁻ subsets of CD8⁺ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L⁻CD8⁺ and/or CD62L⁺CD8⁺ fractions, such as using anti-CD8 and anti-CD62L antibodies.

[0375] In some embodiments, the enrichment for central memory T (T_{CM}) cells is based on positive or high surface expression of CD45RO, CD62L, CCR7, CD28, CD3, and/or CD 127; in some aspects, it is based on negative selection for cells expressing or highly expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8⁺ population enriched for T_{CM} cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CD62L. In one aspect, enrichment for central memory T (T_{CM}) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8⁺ cell population or subpopulation, also is used to generate the CD4⁺ cell population or subpopulation, such that both the positive and negative fractions from the CD4-based separation are retained and used in subsequent steps of the methods, optionally following one or more further positive or negative selection steps.

[0376] In a particular example, a sample of PBMCs or other white blood cell sample is subjected to selection of CD4⁺ cells, where both the negative and positive fractions are retained. The negative fraction then is subjected to negative selection based on expression of CD14 and CD45RA or CD19, and positive selection based on a marker characteristic of central memory T cells, such as CD62L or CCR7, where the positive and negative selections are carried out in either order.

[0377] CD4⁺ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4⁺ lymphocytes can be obtained by standard methods. In some embodiments, naïve CD4⁺ T lymphocytes are CD45RO⁻, CD45RA⁺, CD62L⁺, CD4⁺ T cells. In some embodiments, central memory CD4⁺ cells are CD62L⁺ and CD45RO⁺. In some embodiments, effector CD4⁺ cells are CD62L⁻ and CD45RO⁻.

[0378] In one example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection. For example, in some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinitymagnetic) separation techniques (reviewed in *Methods in Molecular Medicine*, vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior In Vitro and In Vivo, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ).

[0379] In some aspects, the sample or composition of cells to be separated is incubated with small, magnetizable or magnetically responsive material, such as magnetically responsive particles or microparticles, such as paramagnetic beads (e.g., such as Dynalbeads or MACS beads). The magnetically responsive material, e.g., particle, generally is directly or indirectly attached to a binding partner, e.g., an antibody, that specifically binds to a molecule, e.g., surface marker, present on the cell, cells, or population of cells that it is desired to separate, e.g., that it is desired to negatively or positively select.

[0380] In some embodiments, the magnetic particle or bead comprises a magnetically responsive material bound to a specific binding member, such as an antibody or other binding partner. There are many well-known magnetically responsive materials used in magnetic

separation methods. Suitable magnetic particles include those described in Molday, U.S. Pat. No. 4,452,773, and in European Patent Specification EP 452342 B, which are hereby incorporated by reference. Colloidal sized particles, such as those described in Owen U.S. Pat. No. 4,795,698, and Liberti et al., U.S. Pat. No. 5,200,084 are other examples.

[0381] The incubation generally is carried out under conditions whereby the antibodies or binding partners, or molecules, such as secondary antibodies or other reagents, which specifically bind to such antibodies or binding partners, which are attached to the magnetic particle or bead, specifically bind to cell surface molecules if present on cells within the sample.

[0382] In some aspects, the sample is placed in a magnetic field, and those cells having magnetically responsive or magnetizable particles attached thereto will be attracted to the magnet and separated from the unlabeled cells. For positive selection, cells that are attracted to the magnet are retained; for negative selection, cells that are not attracted (unlabeled cells) are retained. In some aspects, a combination of positive and negative selection is performed during the same selection step, where the positive and negative fractions are retained and further processed or subject to further separation steps.

[0383] In certain embodiments, the magnetically responsive particles are coated in primary antibodies or other binding partners, secondary antibodies, lectins, enzymes, or streptavidin. In certain embodiments, the magnetic particles are attached to cells via a coating of primary antibodies specific for one or more markers. In certain embodiments, the cells, rather than the beads, are labeled with a primary antibody or binding partner, and then cell-type specific secondary antibody- or other binding partner (e.g., streptavidin)-coated magnetic particles, are added. In certain embodiments, streptavidin-coated magnetic particles are used in conjunction with biotinylated primary or secondary antibodies.

[0384] In some embodiments, the magnetically responsive particles are left attached to the cells that are to be subsequently incubated, cultured and/or engineered; in some aspects, the particles are left attached to the cells for administration to a patient. In some embodiments, the magnetizable or magnetically responsive particles are removed from the cells. Methods for removing magnetizable particles from cells are known and include, e.g., the use of competing non-labeled antibodies, and magnetizable particles or antibodies conjugated to cleavable linkers. In some embodiments, the magnetizable particles are biodegradable.

[0385] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Auburn, CA). Magnetic Activated Cell Sorting (MACS) systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0386] In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus that carries out one or more of the isolation, cell preparation, separation, processing, incubation, culture, and/or formulation steps of the methods. In some aspects, the system is used to carry out each of these steps in a closed or sterile environment, for example, to minimize error, user handling and/or contamination. In one example, the system is a system as described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1. In one example, the system is a system as described in International Publication Number WO2016/073602.

[0387] In some embodiments, the system or apparatus carries out one or more, e.g., all, of the isolation, processing, engineering, and formulation steps in an integrated or self-contained system, and/or in an automated or programmable fashion. In some aspects, the system or apparatus includes a computer and/or computer program in communication with the system or apparatus, which allows a user to program, control, assess the outcome of, and/or adjust various aspects of the processing, isolation, engineering, and formulation steps.

[0388] In some aspects, the separation and/or other steps is carried out using CliniMACS system (Miltenyi Biotec), for example, for automated separation of cells on a clinical-scale level in a closed and sterile system. Components can include an integrated microcomputer, magnetic separation unit, peristaltic pump, and various pinch valves. The integrated computer in some aspects controls all components of the instrument and directs the system to perform repeated procedures in a standardized sequence. The magnetic separation unit in some aspects includes a

movable permanent magnet and a holder for the selection column. The peristaltic pump controls the flow rate throughout the tubing set and, together with the pinch valves, ensures the controlled flow of buffer through the system and continual suspension of cells.

[0389] The CliniMACS system in some aspects uses antibody-coupled magnetizable particles that are supplied in a sterile, non-pyrogenic solution. In some embodiments, after labelling of cells with magnetic particles the cells are washed to remove excess particles. A cell preparation bag is then connected to the tubing set, which in turn is connected to a bag containing buffer and a cell collection bag. The tubing set consists of pre-assembled sterile tubing, including a pre-column and a separation column, and are for single use only. After initiation of the separation program, the system automatically applies the cell sample onto the separation column. Labelled cells are retained within the column, while unlabeled cells are removed by a series of washing steps. In some embodiments, the cell populations for use with the methods described herein are unlabeled and are not retained in the column. In some embodiments, the cell populations for use with the methods described herein are labeled and are retained in the column. In some embodiments, the cell populations for use with the methods described herein are eluted from the column after removal of the magnetic field, and are collected within the cell collection bag.

[0390] In certain embodiments, separation and/or other steps are carried out using the CliniMACS Prodigy system (Miltényi Biotec). The CliniMACS Prodigy system in some aspects is equipped with a cell processing unit that permits automated washing and fractionation of cells by centrifugation. The CliniMACS Prodigy system can also include an onboard camera and image recognition software that determines the optimal cell fractionation endpoint by discerning the macroscopic layers of the source cell product. For example, peripheral blood is automatically separated into erythrocytes, white blood cells and plasma layers. The CliniMACS Prodigy system can also include an integrated cell cultivation chamber which accomplishes cell culture protocols such as, e.g., cell differentiation and expansion, antigen loading, and long-term cell culture. Input ports can allow for the sterile removal and replenishment of media and cells can be monitored using an integrated microscope. See, e.g., Klebanoff et al. (2012) *J Immunother.* 35(9): 651–660, Terakura et al. (2012) *Blood*.1: 72–82, and Wang et al. (2012) *J Immunother.* 35(9): 689-701.

[0391] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in a fluidic stream. In some embodiments, a cell population described herein is collected and enriched (or depleted) via preparative scale (FACS)-sorting. In certain embodiments, a cell population described herein is collected and enriched (or depleted) by use of microelectromechanical systems (MEMS) chips in combination with a FACS-based detection system (see, e.g., WO 2010/033140, Cho et al. (2010) *Lab Chip* 10, 1567-1573; and Godin et al. (2008) *J Biophoton.* 1(5): 355–376. In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined T cell subsets at high purity.

[0392] In some embodiments, the antibodies or binding partners are labeled with one or more detectable marker, to facilitate separation for positive and/or negative selection. For example, separation may be based on binding to fluorescently labeled antibodies. In some examples, separation of cells based on binding of antibodies or other binding partners specific for one or more cell surface markers are carried in a fluidic stream, such as by fluorescence-activated cell sorting (FACS), including preparative scale (FACS) and/or microelectromechanical systems (MEMS) chips, e.g., in combination with a flow-cytometric detection system. Such methods allow for positive and negative selection based on multiple markers simultaneously.

[0393] In some embodiments, the preparation methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, incubation, and/or engineering. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, e.g., following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1: 1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are generally then frozen to -80°C . at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank.

[0394] In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, cultivation, stimulation, activation, and/or propagation. The incubation and/or engineering may be carried out in a culture vessel, such as a unit, chamber, well, column, tube, tubing set, valve, vial, culture dish, bag, or other container for culture or cultivating cells. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor.

[0395] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, e.g., nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0396] In some embodiments, the stimulating conditions or agents include one or more agent, e.g., ligand, which is capable of activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR, e.g. anti-CD3. In some embodiments, the stimulating conditions include one or more agent, e.g. ligand, which is capable of stimulating a costimulatory receptor, e.g., anti-CD28. In some embodiments, such agents and/or ligands may be, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium (e.g., at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2, IL-15 and/or IL-7. In some aspects, the IL-2 concentration is at least about 10 units/mL.

[0397] In some aspects, incubation is carried out in accordance with techniques such as those described in U.S. Patent No. 6,040,177 to Riddell et al., Klebanoff et al.(2012) J Immunother. 35(9): 651–660, Terakura et al. (2012) Blood.1: 72–82, and/or Wang et al. (2012) J Immunother. 35(9): 689-701.

[0398] In some embodiments, the T cells are expanded by adding to a culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells.

[0399] In some embodiments, the stimulating conditions include temperature suitable for the growth of human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. Optionally, the incubation may further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of about 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least about 10: 1.

IV. COMPOSITIONS

[0400] Also provided are compositions including the binding molecules, such as antibodies, as provided herein, including pharmaceutical compositions and formulations. The compositions and formulations generally include one or more optional acceptable carriers or excipients.

[0401] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0402] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0403] In some aspects, the choice of carrier is determined in part by the particular cell, binding molecule, and/or antibody, and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain

preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0404] Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0405] In some aspects, the composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of

the total composition. Carriers are described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0406] Formulations of the antibodies can include lyophilized formulations and aqueous solutions.

[0407] Compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0408] Sterile injectable solutions can be prepared by incorporating the binding molecule in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives,

preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0409] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0410] The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, colors, and the like. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0411] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

V. METHODS AND USES OF THE ANTIBODIES

[0412] In some embodiments, provided herein are methods involving the use of one or more anti-idiotypic antibodies. In some aspects, provided herein are methods for measuring or detecting a target antibody, such as a CAR or a cell expressing a CAR, and methods for modifying the activity of the target antibody, such as the activity of a CAR or the activity of a cell expressing a CAR. In certain embodiments, the one or more anti-idiotypic antibodies bind, detect, identify, and/or quantify the CAR and/or cells expressing the CAR. In some embodiments, the methods provided herein provide one or more steps of contacting and/or incubating the one or more anti-idiotypic antibodies with a cell or a sample containing or thought to be containing cells that express a chimeric antigen receptor (CAR). In some embodiments, the anti-idiotypic antibody is treated, incubated, and/or contacted with the composition or sample under conditions that allow for the formation of a complex between the anti-idiotypic antibody and the target antibody, e.g., the CAR. In some aspects, the complex may be utilized for the purposes of detecting, isolating, and/or measuring the CAR. In some embodiments, the formation of the complex modifies the activity of the target antibody, e.g., the CAR, such as by

stimulating receptor signaling activity, or in some embodiments, antagonizing the activity of the target antibody, e.g., the CAR, by preventing the association of the CAR with an antigen.

A. Detection/Isolation Methods

[0413] In some embodiments, there are provided methods involving use of one or more of the anti-idiotypic antibodies, and/or molecules (such as conjugates and complexes) containing one or more of such anti-idiotypic antibodies, for detecting, binding, and/or isolating an antibody, e.g., a target antibody. In certain embodiments, the methods provide one or more steps of contacting, incubating, and/or exposing the one or more anti-idiotypic antibodies to a sample and/or composition. In some embodiments, the sample and/or composition has, is likely to have, and/or is suspected of having a target antibody and/or antigen binding fragment thereof that is bound by and/or recognized by the one or more anti-idiotypic antibodies. In certain embodiments, the antibody or antigen binding fragment thereof that is bound by or recognized by the one or more anti-idiotypic antibodies contains one or more fusion domains and/or is a fusion protein. In certain embodiments, the target antibody and/or antigen binding fragment thereof is a CAR. In certain embodiments, the anti-idiotypic antibody, binds to and/or recognizes an anti-CD19 antibody (*e.g.*, antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof, including a chimeric molecule or conjugate including a CAR, containing such anti-CD19 antibody (*e.g.*, antibody fragment).

[0414] The methods in some embodiments include incubating, treating, and/or contacting a sample and/or a composition containing or suspected of containing the target antibody with the anti-idiotypic antibody. In certain embodiments, the incubating is under conditions permissive for binding of the anti-idiotypic antibody to the target antibody present in the composition, for example to form a complex containing the anti-idiotypic antibody and the target antibody.

[0415] In some embodiments, the sample and/or composition contains or is suspected of containing the target antibody, *e.g.*, a CAR. In certain embodiments, the sample and/or composition contains or is suspected of containing cells that express the target antibody, *e.g.*, a CAR. In certain embodiments, the sample is a biological sample. In particular embodiments, the sample is a serum sample or a blood sample. In some embodiments, the biological sample contains one or more immune cells. In some embodiments, the biological sample is or is derived from a tissue, such as connective tissue, muscle tissue, nervous tissue, or epithelial

tissue. In particular embodiments, the biological sample is or is derived from heart, vasculature, salivary glands, esophagus, stomach, liver, gallbladder, pancreas, intestines, colon, rectum, hypothalamus, pituitary gland, pineal gland, thyroid, parathyroid, adrenal gland, kidney, ureter, bladder, urethra, lymphatic system, skin, muscle, brain, spinal cord, nerves, ovaries, uterus, testes, prostate, pharynx, larynx, trachea, bronchi, lungs, diaphragm, bone, cartilage, ligaments, or tendons. In particular embodiments, the biological sample is taken, collected, and/or obtained from a human subject. In certain embodiments, the sample contains cells that are live and/or intact. In some embodiments, the sample is or contains a homogenate and/or cells that have been disrupted and/or lysed. In some embodiments, the biological sample contains proteins and/or antibodies that have been isolated from blood, serum, and/or a tissue.

[0416] In particular embodiments, the anti-idiotypic antibody forms or is capable of forming a complex with a target antibody, e.g., a CAR. In particular embodiments, the complex is detected, measured, quantified, and/or assessed, for example, to allow for the detection, identification, measurement, and/or quantification of the target antibody, for example in a composition or a sample. In certain embodiments, the methods include detecting whether a complex is formed between the anti-idiotypic antibody and the target antibody in the sample, and/or detecting the presence or absence or level of such binding. In some embodiments, the complex contains a detectable label. In particular embodiments, the anti-idiotypic antibody is an immunoconjugate that contains a detectable label. In certain embodiments, the anti-idiotypic antibody contains, is conjugated with, bound to, and/or attached to the detectable label. In some embodiments, the complex contains an antibody that binds to and/or recognizes the anti-idiotypic antibody, e.g., a secondary antibody, that is conjugated with, bound to, and/or attached to a detectable label.

[0417] In some embodiments, methods for detecting, quantifying, detecting, and/or assessing a target antibody, for example in a sample or composition, includes detecting a complex of the target antibody and the anti-idiotypic antibody. In some embodiments, the complex contains a detectable label. In certain embodiments, the complex is probed and/or contacted with a detectable label. In some embodiments, the complex is detected by any suitable method or means, such as but not limited to flow cytometry, immunocytochemistry, immunohistochemistry, western blot analysis, and ELISA.

[0418] In some embodiments, the target antibody or antigen-binding fragment is bound to a cell or expressed on the surface of a cell. In particular embodiments, target antibody, e.g., the CAR is not bound or contained within a cell, for example, in some embodiments, the target antibody is secreted. In certain embodiments, the antibody has been detached, removed, and/or lysed from the surface of a cell.

[0419] In some embodiments, the target antibody or antigen-binding fragment is contained in a chimeric antigen receptor (CAR), such as a CAR expressed on the surface of a cell. In some embodiments, the cell is a stem cell, e.g., an iPSC, or an immune cell. In some embodiments, the immune cell is a T cell, e.g., a CD4⁺ T cell, a CD8⁺ T cell, naïve T (T_N) cell, effector T cell (T_{EFF}), memory T cell, tumor-infiltrating lymphocyte (TIL), immature T cell, mature T cell, helper T cells, cytotoxic T cell, mucosa-associated invariant T (MAIT) cell, naturally occurring and adaptive regulatory T (Treg) cell, helper T cell, such as a TH1 cell, TH2 cell, TH3 cell, TH17 cell, TH9 cell, TH22 cell, follicular helper T cell, alpha/beta T cell, and/or a delta/gamma T cells. In some embodiments, the cell is from a tissue, e.g., heart, vasculature, salivary glands, esophagus, stomach, liver, gallbladder, pancreas, intestines, colon, rectum, hypothalamus, pituitary gland, pineal gland, thyroid, parathyroid, adrenal gland, kidney, ureter, bladder, urethra, lymphatic system, skin, muscle, brain, spinal cord, nerves, ovaries, uterus, testes, prostate, pharynx, larynx, trachea, bronchi, lungs, diaphragm, bone, cartilage, ligaments, or tendons.

[0420] In some embodiments, the target antibody is an anti-CD19 antibody. In some embodiments, the target antibody is or is derived from antibody SJ25C1 or an antigen-binding fragment thereof. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody is or is derived from antibody FMC63 or an antigen-binding fragment thereof. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0421] In some embodiments, there is provided a method of detecting a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof (and/or chimeric molecules comprising such antibody, e.g., antibody fragment, such as a CAR), comprising

contacting a composition comprising the target antibody or antigen-binding fragment with an anti-idiotypic antibody or antigen-binding fragment thereof or an anti-idiotypic antibody immunoconjugate described herein, and detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment. In some embodiments, the method further includes detecting whether a complex is formed between the anti-idiotypic antibody and the target antibody in the composition, such as detecting the presence or absence or level of such binding. In some embodiments, the target antibody or antigen-binding fragment is bound to a cell or expressed on the surface of a cell and the detecting comprises detecting cells bound with the anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is directly or indirectly labeled for detection. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0422] In some embodiments, there is provided a method of isolating a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof (and/or chimeric molecules comprising such antibody, e.g., antibody fragment, such as a CAR), comprising contacting a composition and/or a sample containing or suspected of containing the target antibody or antigen-binding fragment with an anti-idiotypic antibody or antigen-binding fragment thereof or an anti-idiotypic antibody immunoconjugate described herein, and isolating complexes comprising the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment. In certain embodiments, the target antibody is isolated with an anti-idiotypic antibody or antigen binding fragment thereof is an immunoconjugate, such as an immunoconjugate described in Section I-F. In some embodiments, the target antibody or antigen-binding fragment is bound to a cell or expressed on the surface of a cell and the isolating comprises isolating cells bound with the anti-idiotypic antibody. In some embodiments, the complexes comprising the anti-idiotypic antibody are isolated by affinity-based separation. In some embodiments, the affinity-based separation is selected from the group consisting of immunoaffinity-based separation, magnetic-based separation, and affinity chromatography. In some embodiments, the

target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0423] In some embodiments, there is provided a method of detecting a cell expressing a CAR comprising a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising contacting the cell expressing the CAR with an anti-idiotypic antibody or antigen-binding fragment thereof or an anti-idiotypic antibody immunoconjugate described herein, and detecting cells bound with the anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is directly or indirectly labeled for detection. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0424] In certain embodiments, the methods for detecting a target antibody with an anti-idiotypic antibody described herein are used to assess the target antibody in a subject. For example, in some embodiments, provided herein are methods of use for the anti-idiotypic antibody for assessing, measuring, and/or quantifying the *in vivo* pharmacokinetics, expansion, and/or persistence of a CAR expressing cells of a therapeutic cell composition. In some embodiments, the *in vivo* pharmacokinetics, expansion, and/or persistence of the cells, and/or changes in cell phenotypes or functional activity of cells, such as CAR expressing cells administered for immunotherapy, *e.g.* CAR-T cell therapy, in the methods provided herein, can be measured with the anti-idiotypic antibodies provided herein. In some embodiments, the pharmacokinetics, expansion, and/or persistence of the CAR expressing cells are measured, assessed by detecting the presence and/or amount of cells expressing the CAR in the subject and/or in sample obtained from the subject following the administration of the therapeutic cell composition during and/or after the administration of the therapy with an anti-idiotypic antibody provided herein.

[0425] In some aspects, the anti-idiotypic antibody is used with flow cytometry to assess the quantity of cells expressing the recombinant receptor (*e.g.*, CAR-expressing cells administered for T cell based therapy) in the blood or serum or organ or tissue sample (*e.g.*, disease site, *e.g.*, tumor sample) of the subject. In some aspects, persistence is quantified as the number of CAR-expressing cells per microliter of the sample, *e.g.*, of blood or serum, or per total number of peripheral blood mononuclear cells (PBMCs) or white blood cells or T cells per microliter of the sample. In certain aspects, expansion is quantified as the increase in the number of CAR-expressing cells per microliter between samples, *e.g.*, of blood or serum, or per total number of peripheral blood mononuclear cells (PBMCs) or white blood cells or T cells per microliter of the samples over time. In some embodiments, the pharmacokinetics, expansion, and/or persistence are measured or assessed by detecting the amount of CAR expressing cells in the subject and/or in samples collected from the subject at multiple time points. In certain embodiments, the one or more samples are collected, obtained, and/or taken from the subject within 24 hours, 48 hours, 72 hours, 4 days, 5 days, 6 days, 7 days, 10 days, 14 days, 21 days, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 3 months, 4 months, 6 months, one year, or over one year after the therapeutic cell composition is administered.

[0426] In some embodiments, there is provided a method of selecting cells expressing a CAR comprising a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising contacting a population of cells comprising cells expressing the CAR with an anti-idiotypic antibody or antigen-binding fragment thereof or an anti-idiotypic antibody immunoconjugate described herein, and selecting cells bound with the anti-idiotypic antibody. In some embodiments, the cells bound with the anti-idiotypic antibody are selected by affinity-based separation. In some embodiments, the affinity-based separation is selected from the group consisting of immunoaffinity-based separation, flow cytometry, magnetic-based separation, and affinity chromatography. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof or anti-idiotypic antibody immunoconjugate is reversibly bound or immobilized to a support or a stationary phase. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some

embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0427] In some embodiments, there is provided a method of validating a CAR comprising a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising a) incubating a sample comprising T cells transduced with the CAR with an anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR; b) determining the percent of cells bound with the anti-idiotypic antibody or antigen-binding fragment thereof; and c) validating the CAR based on the percent of anti-idiotypic antibody-bound T cells. In some embodiments, the anti-idiotypic antibody is labeled, and anti-idiotypic antibody-bound T cells are assayed by flow cytometry. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0428] Also provided are methods involving use of the provided anti-idiotypic antibodies, and molecules (such as conjugates and complexes) containing one or more of such anti-idiotypic antibodies, for informing treatment decisions in an individual, such as by the detection of CARs recognized by the anti-idiotypic antibody, such as CARs comprising a target antibody, such as an anti-CD19 antibody (*e.g.*, antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof. In some embodiments, the methods are for informing treatment decisions in an individual in association with a therapy comprising administration of CAR T cells, such as anti-CD19 CAR T cells. The methods in some embodiments include incubating and/or probing a biological sample with the anti-idiotypic antibody and/or administering the anti-idiotypic antibody to the individual. In certain embodiments, a biological sample includes a cell or tissue or portion thereof, such as tumor or cancer tissue or biopsy or section thereof. In certain embodiments, the incubating is under conditions permissive for binding of the anti-idiotypic antibody to CARs present in the sample. In some embodiments, the methods further include detecting whether a complex is formed between the anti-idiotypic antibody and CARs in the sample, such as detecting the

presence or absence or level of such binding. Such a method may be an in vitro or in vivo method.

[0429] In one embodiment, an anti-idiotypic antibody is used to determine whether adjustment to a CAR T cell therapy in an individual is necessary, e.g. where low levels of the CAR T cells in the individual indicate the need to adjust the therapy. In some embodiments, the target antibody is an anti-CD19 antibody. In some embodiments, the target antibody is or is derived from antibody SJ25C1 or an antigen-binding fragment thereof. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody is or is derived from antibody FMC63 or an antigen-binding fragment thereof. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0430] In some embodiments, there is provided a method of evaluating a CAR T cell therapy in an individual, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising incubating a sample from the individual with an anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR and determining the amount of T cells bound with the anti-idiotypic antibody, and determining the potential therapeutic benefit of the therapy based on the amount of anti-idiotypic antibody-bound T cells. In some embodiments, the anti-idiotypic antibody is labeled, and anti-idiotypic antibody-bound T cells are assayed by flow cytometry. In some embodiments, the sample is a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0431] In some embodiments, there is provided a method of evaluating a CAR T cell therapy in an individual. In some aspects, the CAR comprises a target antibody, such as an antibody that is or is derived from SJ25C1 or FMC63, such as an antigen-binding fragment of full-length

SJ25C1 or FMC63. In some embodiments, the method includes administering an anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR (e.g., targeting the antibody, e.g., antibody fragment, of the CAR) to the individual. In some aspects, the administration is carried out following initiation of a first dose of the therapy. The method can comprise determining the presence of the anti-idiotypic antibody in one or more tissues/organs in the individual. In some aspects, the method includes determining the potential therapeutic benefit of the therapy based on the presence of the anti-idiotypic antibody in at least one of the one or more tissues/organs. In some embodiments, the anti-idiotypic antibody is labeled, and presence of the anti-idiotypic antibody in the individual is determined by imaging in the individual to detect the label. In some embodiments, determining the presence of the anti-idiotypic antibody in one or more tissues/organs in the individual includes or is carried out by determining a level of the anti-idiotypic antibody or binding thereof in the one or more tissues/organs.

[0432] In some embodiments, the method further comprises administering the anti-idiotypic antibody to the individual following initiation of a second or subsequent dose of the therapy and/or determining the presence of the anti-idiotypic antibody in the one or more tissues/organs in the individual. In some aspects, the method further involves determining the potential therapeutic benefit of the therapy based on the difference in the level of the anti-idiotypic antibody in at least one of the one or more tissue/organs in the individual between the first and second anti-idiotypic antibody administrations. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0433] In some embodiments, there is provided a method of adjusting a CAR T cell therapy in an individual, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof. In some aspects, the method includes incubating or contacting a sample from the individual with an anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR. In some aspects, the method includes determining the amount of T cells bound with or to the anti-idiotypic antibody. In some aspects, the method includes adjusting the therapy based on the amount of anti-idiotypic antibody-bound T cells. In

some embodiments, the anti-idiotypic antibody is labeled directly or indirectly. In some aspects of such embodiments, the anti-idiotypic antibody-bound T cells are imaged in vivo or ex vivo, such as, in some cases, by assaying a sample, from the subject administered the CAR-T cells and anti-idiotypic antibody, by flow cytometry. In some embodiments, the sample is a blood-derived sample, and/or is or is derived from an apheresis or leukapheresis product. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0434] In some embodiments, there is provided a method of adjusting a CAR T cell therapy in an individual. In some aspects, such a method is carried out for a CAR-T cell therapy where the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, including an antigen-binding fragment of SJ25C1 or FMC63. The method in some embodiments comprises administering, to the individual, an anti-idiotypic antibody or antigen-binding fragment thereof that targets or binds the CAR following initiation of administration of a first dose of the therapy and determining the presence, absence or level of the anti-idiotypic antibody in one or more tissues/organs in the individual. In some aspects, the method includes adjusting the therapy based on the presence, absence or level of the anti-idiotypic antibody in at least one of the one or more tissues/organs. In some embodiments, the anti-idiotypic antibody is labeled directly or indirectly, and in some such embodiments the presence of the anti-idiotypic antibody in the individual is determined by imaging in the individual to detect the label. In some embodiments, determining the presence of the anti-idiotypic antibody in one or more tissues/organs in the individual comprises determining a level of or for the anti-idiotypic antibody in the one or more tissues/organs or binding thereof. In some embodiments, the method further comprises administering the anti-idiotypic antibody to the individual after initiation of a second or subsequent dose of administration of the therapy and in some aspects determining the presence, absence or level of the anti-idiotypic antibody in the one or more tissues/organs in the individual, and/or adjusting the therapy based on the observations such as based on the difference in the level of the anti-idiotypic antibody in at least one of the one or more tissue/organs in the

individual between the first and second anti-idiotypic antibody administrations. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0435] In some aspects, the therapy is adjusted (i) if the number of cells of the T cell therapy detectable in the blood or other biological sample, after having been detectable, is not detectable or is reduced, optionally reduced compared to a preceding time point after administration of the T cell therapy; (ii) the number of cells of the T cell therapy detectable in the blood or other biological sample is decreased by or more than 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold or more the peak or maximum number cells of the T cell therapy detectable in the blood or the biological sample of the subject after initiation of administration of the T cell therapy, optionally the first, second or subsequent dose; (iii) at a time after a peak or maximum level of the cells of the T cell therapy are detectable in the blood of the subject, the number of cells of or derived from the T cells detectable in the blood from the subject is less than less than 10%, less than 5%, less than 1% or less than 0.1% of total peripheral blood mononuclear cells (PBMCs) in the blood of the subject; and/or (iv) if the number of CD3+ or CD8+ cells of the cell therapy detectable in the blood is less than 20 cells per μL , 15 cells per μL , 10 cells per μL , less than 5 cells per μL or less than 1 cells per μL . In some embodiments, the therapy is adjusted by administered one or more additional doses of the CAR-T cell therapy, administered an increased dose of the CAR-T cell therapy, administering an alternative CAR-T cell therapy specific for the same or different antigen, administering one or more immunomodulatory agent or other agent for promoting or increasing expansion or persistence of the CAR-T cells.

[0436] Various methods known in the art for detecting specific antibody-antigen binding can be used. Exemplary immunoassays include fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA). An indicator moiety, or label group, can be attached to the anti-idiotypic antibodies and is selected so as to meet the needs of various uses of the method which are often dictated by the

availability of assay equipment and compatible immunoassay procedures. Exemplary labels include radionuclides (*e.g.* ^{125}I , ^{131}I , ^{35}S , ^3H , or ^{32}P and/or chromium (^{51}Cr), cobalt (^{57}Co), fluorine (^{18}F), gadolinium (^{153}Gd , ^{159}Gd), germanium (^{68}Ge), holmium (^{166}Ho), indium (^{115}In , ^{113}In , ^{112}In , ^{111}In), iodine (^{125}I , ^{123}I , ^{121}I), lanthanum (^{140}La), lutetium (^{177}Lu), manganese (^{54}Mn), molybdenum (^{99}Mo), palladium (^{103}Pd), phosphorous (^{32}P), praseodymium (^{142}Pr), promethium (^{149}Pm), rhenium (^{186}Re , ^{188}Re), rhodium (^{105}Rh), ruthenium (^{97}Ru), samarium (^{153}Sm), scandium (^{47}Sc), selenium (^{75}Se), (^{85}Sr), sulphur (^{35}S), technetium (^{99}Tc), thallium (^{201}Tl), tin (^{113}Sn , ^{117}Sn), tritium (^3H), xenon (^{133}Xe), ytterbium (^{169}Yb , ^{175}Yb), yttrium (^{90}Y), enzymes (*e.g.*, alkaline phosphatase, horseradish peroxidase, luciferase, or β -galactosidase), fluorescent moieties or proteins (*e.g.*, fluorescein, rhodamine, phycoerythrin, GFP, or BFP), or luminescent moieties (*e.g.*, QdotTM nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, Calif.). Various general techniques to be used in performing the various immunoassays noted above are known.

[0437] In some embodiments, anti-idiotypic antibodies need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to any of the anti-idiotypic antibodies.

[0438] The anti-idiotypic antibodies provided herein can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp. 147-158 (CRC Press, Inc. 1987).

[0439] The anti-idiotypic antibodies can also be used for *in vivo* diagnostic assays, such as *in vivo* imaging. Generally, the anti-idiotypic antibody is labeled with a radionuclide (such as ^{111}In , ^{99}Tc , ^{14}C , ^{131}I , ^{125}I , or ^3H) so that the cells or tissue of interest can be localized *in vivo* following administration to an individual.

[0440] In some embodiments, the anti-idiotypic antibody or antigen-binding fragment is immobilized or bound to a solid support, wherein one or more target cells comprising CAR-T cells are contacted with the solid support. In some embodiments, the solid support is a bead. In some embodiments, the solid support is the surface of a well or plate, *e.g.*, a cell culture plate. In some embodiments, the solid support is a resin or matrix present in or contained within a chromatography column, for example, to permit chromatographic isolation or selection of CAR+

T cells. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment is or is capable of being reversibly bound to a solid support. In some embodiments, the solid support is an affinity chromatography matrix comprising one or more binding sites capable of binding, e.g. reversibly binding, to a binding partner present in the anti-idiotypic antibody. In one exemplary embodiment, the anti-idiotypic antibody comprises a streptavidin-binding peptide or other streptavidin binding moiety capable of binding to a streptavidin or streptavidin mutein molecule present on or immobilized on the solid support, which, in some cases, can be dissociated in the presence of a competition substance, such as biotin. Exemplary of such systems include those described in U.S. published patent application No. US20150024411.

[0441] In some aspects, the anti-ID antibodies provided herein can be expressed on the surface of a cell. In some aspects, a cell-expressed anti-ID antibody can be used to induce or stimulate a CAR-expressing cell, such as part of a system for selectively growing CAR cells. In some aspects, the anti-ID antibody or antigen-binding fragment thereof is expressed on an artificial antigen presenting cell (aAPC). The anti-ID-expressing aAPCs can be used as reagents for stimulation or expansion of CAR T-cell populations.

[0442] Methods for preparing or generating aAPCs are known, see e.g. U.S. Pat. Nos. 6,225,042, 6,355,479, 6,362,001, 6,790,662; 7,754,482; U.S. Patent Application Publication Nos. 2009/0017000 and 2009/0004142; and International Publication No. WO2007/103009. Various aAPCs are known in the art, see e.g., U.S. Patent No. 8,722,400, published application No. US2014/0212446; Butler and Hirano (2014) *Immunol Rev.*, 257(1):10. 1111/imr.12129; Suhoshki et al. (2007) *Mol. Ther.*, 15:981-988).

[0443] aAPCs include features of natural APCs, including expression of an MHC molecule, stimulatory and costimulatory molecule(s), Fc receptor, adhesion molecule(s) and/or the ability to produce or secrete cytokines (e.g. IL-2). Normally, an aAPC is a cell line that lacks expression of one or more of the above, and is generated by introduction (e.g. by transfection or transduction) of one or more of the missing elements necessary for stimulation of a cell, e.g. a CAR-T cell.

[0444] In some embodiments, cells selected to become aAPCs have deficiencies in intracellular antigen-processing, intracellular peptide trafficking, and/or intracellular MHC Class I or Class II molecule-peptide loading. In some aspects, such cells also lack the ability to

express an MHC Class I or Class II molecule and/or molecules involve in or related to antigen processing. Exemplary aAPCs either constitute or are derived from a transporter associated with antigen processing (TAP)-deficient cell line, such as an insect cell line. An exemplary cell line is a *Drosophila* cell line, such as a Schneider 2 cell line (see, e.g. Schneider, J. Embryol. Exp. Morph. 1972 Vol 27, pp. 353-365). Illustrative methods for the preparation, growth, and culture of Schneider 2 cells, are provided in U.S. Pat. Nos. 6,225,042, 6,355,479, and 6,362,001.

[0445] In some embodiments, the cell is a K652 cell or a K562-derived cell. In some embodiments, the cell is the cell line available at ATCC No. CCL-243.

[0446] In some aspects, the aAPC is further engineered to express a further molecule to enhance, potentiate or augment stimulation of a CAR-expressing T cell. In some embodiments, the further molecule is an immune stimulatory ligand, a co-stimulatory ligand, a cytokine or an adhesion molecule. In some embodiments, the co-stimulatory ligand specifically binds with at least one co-stimulatory molecule present on a T cell. In some embodiments, the aAPC is generated, e.g. by transduction or transfection, to express one or more of a co-stimulatory signal (e.g. CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, ICOS-L, ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, ILT3, ILT4, 3/TR6 or a ligand of B7-H3; or an antibody that specifically binds to CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, LIGHT, NKG2C, B7-H3, Toll ligand receptor or a ligand of CD83), a cell adhesion molecule (e.g. ICAM-1 or LFA-3) and/or a cytokine (e.g. IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-21, interferon-alpha (IFN α), interferon-beta (IFN β), interferon-gamma (IFN γ), tumor necrosis factor-alpha (TNF α), tumor necrosis factor-beta (TNF β), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (GCSF)). In some cases, an aAPC does not normally express an MHC molecule, but can be engineered to express an MHC molecule or, in some cases, is or can be induced to express an MHC molecule, such as by stimulation with cytokines. In some cases, aAPCs also can be loaded with a stimulatory or co-stimulatory ligand, which can include, for example, an anti-CD3 antibody, an anti-CD28 antibody or an anti-CD2 antibody. In some embodiments, the aAPC expresses a molecule capable of mediating a primary signal in the cell, such as mediated via the T cell receptor/CD3 complex on a T cell. In some embodiments,

the aAPCs comprise a stimulatory ligand that specifically binds with a TCR/CD3 complex such that a primary signal is transduced.

[0447] In some embodiments, because the anti-ID is capable of delivering a signal through the CAR, the aAPC does not express a stimulatory ligand that specifically binds with a TCR/CD3 complex. In some embodiments, the aAPC does not express a costimulatory molecule.

[0448] In some embodiments, the anti-ID is expressed as a single chain fragment (scFv) for expression on the surface of the cell. The nucleic acid encoding the scFv can be fused to DNA sequences encoding a transmembrane domain. Transmembrane regions of particular use include those derived from the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In some cases, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Exemplary transmembrane domain include those derived from CD8 or CD28.

B. Use in Cell Stimulation

[0449] In some embodiments, the provided anti-idiotypic antibodies or antigen-binding fragments thereof are agonists and/or exhibit specific activity to stimulate cells expressing a target antibody including conjugates or chimeric receptors containing the same, such as an anti-CD19 antibody (*e.g.*, antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof. In some embodiments, provided are methods involving use of the provided anti-idiotypic antibodies, and molecules (such as conjugates and complexes) containing one or more of such anti-idiotypic antibodies, for stimulation or activation of CAR-expressing or other chimeric receptor-expressing cells, such as T cells. In some aspects, the CAR or other receptor comprises the target antibody, such as an anti-CD19 antibody (*e.g.*, antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof.

[0450] In some embodiments, the methods can be used in connection with methods of preparing genetically engineered T cells, such as in methods of expanding genetically engineered T cells or other cells into which a nucleic acid molecule encoding the chimeric

receptor such as the CAR comprising the target antibody has been introduced, e.g., by transfection, transduction, or a non-viral means of nucleic acid transfer, such as transposon-based approaches. In some aspects, the target antibody is an anti-CD19 antibody (e.g., antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof. In particular embodiments, the target antibody is or contains a CAR, e.g., an anti-CD19 CAR. In particular embodiments, the anti-CD19 CAR contains an scFv that is from and/or is derived from an anti-CD19 antibody such as antibody SJ25C1 or FMC63.

[0451] The methods in some embodiments include incubating a sample comprising T cells transduced with a CAR with the anti-idiotypic antibody. In certain embodiments, the methods further include detecting whether the CAR T cells are activated or stimulated, such as by assessing the viability, proliferation, and/or expression of activation markers in the CAR T cells. In some embodiments, the target antibody is an anti-CD19 antibody. In some embodiments, the target antibody is or is derived from antibody SJ25C1 or FMC63 or an antigen-binding fragment thereof. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0452] In some embodiments, there is provided a method of simulating cells, comprising incubating an input composition comprising cells expressing a CAR comprising a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, with an anti-idiotypic antibody or antigen-binding fragment thereof described herein, thereby generating an output composition comprising stimulated cells. In some embodiments, the incubation is performed under conditions in which the anti-idiotypic antibody or antigen-binding fragment thereof binds to the CAR, thereby inducing or modulating a signal in one or more cells in the input composition. In some embodiments, the cells comprise T cells. In some embodiments, the T cells comprise CD4+ and/or CD8+ T cells.

[0453] In some embodiments, provided herein is a method of stimulating or expanding cells that express a CAR, by incubating an input composition containing cells expressing a CAR with an anti-ID antibody that binds to and/or recognizes the CAR. In some embodiments, binding

between the anti-ID antibody and the CAR induces expansion of the cells expressing the CAR, thereby producing an output composition comprising expanded cells.

[0454] In some embodiments, anti-idiotypic antibody is contacted to or incubated with an input composition of one or more cells to generate an output composition. In certain embodiments, the input cells and/or the input composition is a composition and/or a plurality of cells that are, or are desired to be, treated, incubated, or contacted under conditions that will produce one or more changes to at least a portion of the cells of the input composition, thereby converting the input composition into an output composition. In some embodiments, the input cells are a composition of immune cells, for example, a composition of T cells that contain cells expressing a CAR. In particular embodiments, at least a portion of the cells in the input composition are activated, expanded, and/or enriched in the generated output composition by practice of the provided methods.

[0455] In certain embodiments, the anti-idiotypic antibody expands or enriches the CAR expressing cells of an input composition. In some embodiments, the input composition comprises eukaryotic cells, such as mammalian cells. In certain embodiments, the input composition contains human cells. In some embodiments, the input composition contains cells that are derived from the blood, bone marrow, lymph, or lymphoid organs. In particular embodiments, the input composition contains cells of the immune system, i.e., cells of innate or adaptive immunity, e.g., myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. In some embodiments, the input composition contains stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). In particular embodiments, the input composition contains CD3⁺ cells. In certain embodiments, the input composition contains CD4⁺ cells. In some embodiments, the input composition contains CD8⁺ cells. In some embodiments, the input composition is a composition of CD4⁺ cells. In particular embodiments, the input composition is a composition of CD8⁺ cells.

[0456] In some embodiments, the methods and agents are capable of stimulating T cells deficient in or that have downregulated one or more natural signaling molecules such as one or more costimulatory receptors or antigen receptors or cytokine receptors but that express the chimeric receptor, e.g., the CAR, recognized by the anti-Id antibody. In some embodiments, cells of the input composition are low or negative for surface expression of CD28 or other

costimulatory molecule or other signaling molecule. Thus in some embodiments, the provided agents and methods have certain advantages compared to certain other activation or stimulatory agents or methods that which may require or depend upon surface expression of CD28 or other endogenous signaling molecule, to provide the desired signal and/or the full extent of such signal, e.g., to provide costimulatory signal and/or to achieve full activation/ In some embodiments, the provided agents and methods are advantageous in such regards compared to anti-CD3/anti-CD28 reagents (e.g. beads); in some aspects, th the provided anti-ID antibodies are advantageous in being able to stimulate or achieve a desired effect such as activation or proliferation of cells that are low or negative for CD28 or other natural signaling molecule. In some aspects, signaling through the CAR by stimulation with an anti-ID antibody results in both a primary and secondary (costimulatory) signal via the CAR using only the single reagent. In some embodiments, the input composition comprises CD3+ cells that express low levels of CD28 or other endogenous signaling molecule. In some embodiments, the input composition comprises CD3+ cells that are CD28 negative or are negative for other endogenous signaling molecule. In some embodiments, the anti-ID antibody stimulates activation and/or expansion of cells expressing low levels of CD28 or cells that are CD28 negative. In some embodiments, the cells are contacted with anti-idiotypic antibody or antigen-binding fragment that is immobilized or bound to a solid support. In some embodiments, the solid support is a bead. In some embodiments, the solid support is the surface of a well or plate, e.g., a cell culture plate. In some examples, the anti-ID antibody is soluble. In certain embodiments, the cells are not contacted with anti-CD3/anti-CD28 conjugated reagents prior to contacting the cells with the anti-idiotypic antibody or antigen-binding fragment.

[0457] In certain embodiments, the anti-idiotypic antibody is applied to, contacted to, or incubated with an input composition of cells that have been transduced or transfected with a nucleotide encoding a CAR. In particular embodiments, incubating, treating, and/or contacting input cells with the anti-idiotypic antibody results in an expansion and/or enrichment of cells expressing the CAR. In particular embodiments, incubating, treating, and/or contacting input cells with the anti-idiotypic antibody does not result in an expansion and/or enrichment of cells that do not express the CAR. In particular embodiments, incubating, treating, and/or contacting input cells with the anti-idiotypic antibody results in an expansion and/or enrichment of cells that

do not express the CAR that is at least 50%, at least 75%, at least 85%, at least 90%, at least 95%, at least 99%, at least 99.9% or at least 99.99% less than the expansion and/or enrichment of cells that express the CAR. In some embodiments, the anti-idiotypic antibodies provided herein are used to expand CAR expressing cells of an input composition that experienced a low transduction and/or transfection efficiency, and/or that contains a low amount CAR expressing cells. In certain embodiments, the anti-idiotypic antibody selectively expands and/or enriches cells that express a CAR.

[0458] Some embodiments contemplate that the anti-idiotypic antibody is more effective for expanding and/or enriching cells of an input composition with a low transduction or transfection efficiency and/or have a low amount of cells that express the CAR than by expanding and/or enriching the cells by polyclonal stimulation, e.g., anti-CD3 and/or anti-CD28 antibody stimulation. In particular embodiments, polyclonal stimulation results in expansion of cells that express and cells that do not express the CAR in the input composition, and therefore, in some embodiments, may fail to enrich CAR expressing cells, particular when the input composition has a low number of CAR expressing cells. In contrast, in some embodiments, incubation with an anti-idiotypic antibody results in a selective expansion of CAR expressing cells and will therefore, in certain embodiments, result in selective expansion and/or enrichment of the CAR expressing cells. In some embodiments, incubating, contacting, and/or treating input cells with the anti-idiotypic antibody results in a greater enrichment and/or expansion of CAR expressing cells than by polyclonal stimulation.

[0459] In particular embodiments, the anti-idiotypic antibody is incubated with, applied to, and/or contacted with input cells that were transfected and/or transduced with a lower amount of viral particles, ratio of copies of the viral vector particles to cells, and/or infectious units (IU), than input cells that are expanded and/or enriched by polyclonal stimulation. For example, in some embodiments, the input composition that is incubated with the anti-idiotypic antibody is generated from cells that were transduced with or with at least 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or 60 fewer IU per cell than the input composition that is expanded and/or enriched by polyclonal stimulation. In some embodiments, the input composition that is incubated with the anti-idiotypic antibody is generated from cells that were transduced with a titer of viral vector particles with or with at least 1×10^5 IU/mL, 5×10^5 IU/mL, 1×10^6 IU/mL, 5×10^6 IU/mL, $6 \times$

10^6 IU/mL, 7×10^6 IU/mL, 8×10^6 IU/mL, 9×10^6 IU/mL, or 1×10^7 IU/mL less than the input composition that is expanded and/or enriched by polyclonal stimulation.

[0460] In particular embodiments, transducing cells with a high IU/cell will lead to high transduction efficiency but, in some embodiments, may also lead to transfected cells with a high vector copy number (VCN), which can present safety risks and may not meet regulatory standards. In particular embodiments, lowering the IU/cell that cells are transduced with will reduce transduction efficiency but will lower VCN. In particular embodiments, increasing the IU/cell that cells are transduced with will increase transduction efficiency but will also increase VCN.

[0461] In some embodiments, an input composition contains a population of cells that have been transduced or transfected, or cells that are derived from cells that have been transduced or transfected, with one or more nucleic acids encoding a CAR, that is bound by or recognized by the anti-idiotypic antibody. In some embodiments, the input composition contains less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, or less than 1% of the cells are CAR expressing cells. In particular embodiments, the cells from the input composition have been transfected or transduced as described in Section III. In certain embodiments, the input cells contains a population of cells that have been transduced or transfected, or cells that are derived from cells that have been transduced or transfected, with one or more nucleic acids encoding an anti-CD19 CAR, such as an anti-CD19 CAR that contains an scFv that is from and/or is derived from an anti-CD19 antibody such as antibody SJ25C1 or FMC63.

[0462] In particular embodiments, the incubation, contacting, or treatment of cells from the input composition with the anti-idiotypic antibody is performed under conditions for stimulation, expansion, and/or activation of cells which conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, e.g., nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0463] In some embodiments, the cells of the input composition have been transfected or transduced with one nucleic acid comprising a gene encoding a CAR and the cells are contacted, incubated, or treated with the anti-idiotypic antibody that binds to or recognizes the recombinant receptor. In some embodiments, the cells of the input composition are treated, incubated, or contacted with the anti-idiotypic antibody after the cells transduced or transfected nucleic acid encoding a CAR. In particular embodiments, the cells of the input composition are treated, incubated, or contacted with the anti-idiotypic antibody immediately, within about 1 minute, within about 5 minutes, within about 30 minutes, within about 1 hour, within about 2 hours, within about 4 hours, within about 6 hours, within about 8 hours, within about 12 hours, within about 24 hours, within about 2 days, within about 3 days, within about 4 days, within about 5 days, within about 6 days, within about 1 week, within about 2 weeks, within about 3 weeks, within about 4 weeks, within about 5 weeks, or within about 6 weeks after the cells of the input composition have been transduced or transfected.

[0464] In some embodiments, cells of the input composition are treated, incubated, and/or contacted with soluble anti-idiotypic antibody, contacted with an antibody that is not crosslinked and/or contacted with an antibody that is not bound or attached to a solid support

[0465] In some embodiments, the methods result in proliferation, activation, stimulation, cytokine release, or other functional outcome such as upregulation of an activation marker or cytokine release or production, of cells expressing the chimeric receptor such as the CAR recognized by the anti-Id antibody. In some aspects, such proliferation or other functional response or readout is induced in such cells to a degree that is similar to or greater than that induced by incubation of the cells with an agent and/or conditions that stimulates proliferation of T cells, such as anti-CD3/CD28 beads and/or crosslinked anti-CD3. In some aspects, the methods do not involve crosslinking of the anti-idiotypic antibody. In some aspects of any of the embodiments, the anti-idiotypic agents are capable of inducing the specified proliferation or functional outcome or degree thereof, without crosslinking of the anti-idiotypic antibody. In some aspects, anti-idiotypic agents herein are advantageous in their ability to stimulate or cause a particular functional outcome of T cells or other immune cells expressing the target receptor, without the need to crosslink the anti-Id antibody or use a secondary agent. In some aspects, the

result is achieved with soluble or plate-bound form of the anti-idiotypic antibody. In some aspects, the result is achieved with the anti-idiotypic antibody coupled to a bead.

[0466] . In particular embodiments, the cells of the input composition are treated, incubated, and/or contacted with between 10 pg/ml and 100 µg/ml, between 1 pg/ml and 1 ng/ml, between 1 ng/ml and 1 µg/ml between 100 ng/ml and 1.0 µg/ml, between 1 ng/ml and 100 ng/ml, between 10 ng/ml and 1.0 µg/ml, between 100 ng/ml and 10 pg/ml, between 250 ng/ml and 10 µg/ml, between 250 pg/ml and 1 ng/ml, between 1 µg/ml and 10 µg/ml, between 250 ng and about 2.5 µg/ml, or between 1 µg/ml and 10 µg/ml.

[0467] In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a solid support, which optionally comprises or is conjugated to a reagent comprising a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof. In some embodiments, the solid support is a surface of a plate or a well. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a soluble reagent, which optionally is or comprises a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof. In some embodiments, the reagent comprises a streptavidin mutein. In one exemplary embodiment, the anti-idiotypic antibody comprises a streptavidin-binding peptide or other streptavidin binding moiety capable of binding to a streptavidin or streptavidin mutein molecule present on or immobilized on the soluble reagent, which, in some cases, can be dissociated in the presence of a competition substance, such as biotin. Exemplary of such systems include those described in PCT published patent application No. WO2015/158868.

[0468] In particular embodiments, the cells of the input composition are treated, incubated, and/or contacted with anti-idiotypic antibody that is attached, bound, coated, and/or conjugated to a solid surface or support, e.g., a plate or a well. In certain embodiments, the anti-idiotypic antibody has been attached, bound, coated, and/or conjugated to the solid surface or support by incubating the solid surface or support with a concentration of the anti-idiotypic antibody. In particular embodiments, the solid surface or support is incubated with between 10 ng/ml and 100 µg/ml, between 100 ng/ml and 1.0 µg/ml, between 250 ng/ml and 10 µg/ml, between 250 ng/ml and 1 µg/ml, between 1 µg/ml and 10 µg/ml, between 250 ng and 2.5 µg/ml, or between 1 µg/ml and 10 µg/ml the anti-idiotypic antibody. In some embodiments, the solid surface or support is

incubated with between 250 ng/ml and 10 µg/ml. In certain embodiments, the solid surface or support is incubated with or with about 0.25 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 1.25 µg/ml, 2 µg/ml, 2.5 µg/ml, 5 µg/ml or 10 µg/ml the anti-idiotypic antibody.

[0469] In some embodiments, the incubation is for at least or about at least 5 minutes, 10 minutes, 30 minutes, 60 minutes, 2 hours, 6 hours, 12 hours, 24 hours, 36, 48 hours, 72 hours or 96 hours. In some embodiments, the input composition comprises less than or less than about 60%, less than or less than about 50%, less than or less than about 40%, less than or less than about 30%, less than or less than about 20% or less than or less than about 10% CAR-expressing cells as a percentage of the total cells in the composition. In some embodiments, the number of CAR-expressing cells in the output composition is increased by greater than 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold or more compared to the number of CAR-expressing cells in the input composition; and/or the percentage of CAR-expressing in the output composition compared to the total cells in the composition is increased by greater than 10 %, 20 %, 40 %, 50 %, 60 %, 70 %, 80 % or more. In some embodiments, prior to incubating, the cells are not selected or enriched for CAR-expressing cells. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0470] In certain embodiments, the anti-idiotypic antibody are contacted or incubated with cells from the input composition, e.g. comprising cells that express a CAR, for an amount of time to expand one or more cells of the input composition, such as to expand cells of the input composition that express the recombinant receptor. In particular embodiments, the cells from the input composition are contacted, incubated, or treated with the anti-idiotypic antibody for at least about 12 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 3 weeks, or at least about 4 weeks. In particular embodiments, the cells from the input composition are contacted, incubated, or treated with the anti-idiotypic antibody for less than about 1 day, less than about 2 days, less than about

3 days, less than about 4 days, less than about 5 days, less than about 6 days, or less than or about 12 days. In some embodiments, the cells from the input composition are contacted, incubated, or treated with the anti-idiotypic antibody for between about 1 day and about 14 days, between about 3 days, and 7 days, or for between 4 days and 6 days.

[0471] In particular embodiments, cells from an input composition, e.g. comprising cells that express a CAR, are incubated, contacted, or treated with anti-idiotypic antibody at temperatures greater than room temperature to expand the cells of the input composition that express the recombinant receptor. In some embodiments, the treatment, incubation, or contacting is performed at a temperature greater than about 25 °C, such as generally greater than or greater than about 32 °C, 35 °C or 37 °C. In some embodiments, the treatment, contacting, or incubation is performed at a temperature of at or about 37 °C $\pm$ 2 °C, such as at a temperature of at or about 37 °C.

[0472] In some embodiments, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, at least 99.9%, about 100%, or 100% of the cells of the output composition express the CAR.

[0473] In particular embodiments, the number of cells that express the CAR in the output composition that was incubated, treated, and/or contacted with the anti-idiotypic antibody is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 100%, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or at least 100-fold greater than the number of cells that express the CAR in the input composition.

[0474] In particular embodiments, the percentage of cells that express the CAR in the output composition that was incubated, treated, and/or contacted with the anti-idiotypic antibody is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 100%, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold,

at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or at least 100-fold greater than the number of cells that express the CAR in the input composition.

[0475] In some embodiments, the number of cells that express the CAR in the output composition that was incubated, treated, and/or contacted with the anti-idiotypic antibody is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 100%, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or at least 100-fold greater than the number of cells of an output composition that received polyclonal stimulation, incubation with anti-CD3 and anti-CD28 antibodies.

[0476] In certain embodiments, the percentage of cells that express the CAR in the output composition that was incubated, treated, and/or contacted with the anti-idiotypic antibody is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 100%, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or at least 100-fold greater than the number of cells of an output composition that received polyclonal stimulation, incubation with anti-CD3 and anti-CD28 antibodies.

[0477] In some embodiments, the cells that express the CAR in the output composition that was incubated, treated, and/or contacted with the anti-idiotypic antibody contain at least a 1%, at least a 5%, at least a 10%, at least a 15%, at least a 20%, at least a 25%, at least a 30%, at least a 35%, at least a 40%, at least a 45%, at least a 50%, at least a 55%, at least a 60%, at least a 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, or at least a 99% lower VCN than cells of an output composition that received polyclonal stimulation, e.g., incubation with anti-CD3 and anti-CD28 antibodies. In some embodiments, the average VCN of CAR expressing cells of the output no more than at or about 10, 5, 4, 2.5, 1.5, or 1.

[0478] In some embodiments, such methods can be used as part of the manufacturing, analytic, and/or quality control methods, e.g., in association with the generation of cell therapies expressing recombinant polypeptides containing an antibody or fragment thereof recognized by the anti-idiotypic antibody, such as the CAR T cells, for testing purpose, including to test expression and/or potency of the engineered receptor, e.g., in cells engineered for use in therapy in an individual. In certain embodiments, the cell compositions may be tested at any stage in the process of generating CAR expressing T cells. In particular embodiments, a sample of cells may be collected from a cell composition at any stage of the process and stored, e.g., by cryofreezing and/or cryopreservation, for later testing and/or analysis. The compositions tested may be pharmaceutical compositions e.g., including those containing the cells and a pharmaceutically acceptable recipient and/or cryopreservative agent.

[0479] In some embodiments, the anti-idiotypic antibody stimulates cells expressing a target antibody, e.g., a CAR, *in vivo*. Particular embodiments contemplate that CAR-T cell therapies are effective in the treatment of cancer and other diseases and disorders. However, in certain contexts, available approaches to CAR-T cell therapy may not always be entirely satisfactory. For example, in some embodiments, the exposure and persistence of CAR expressing cells in the subject is reduced or declines over time. Yet, observations indicate that, in some cases, increased exposure of the CAR expressing cells may improve efficacy and therapeutic outcomes in CAR-T cell therapy. Thus, in some embodiments, the anti-idiotypic antibody is administered to boost, augment and/or increase persistence and/or expansion of CAR expressing cells.

[0480] In certain embodiments, the anti-idiotypic antibody is administered to a subject, such as a subject who has previously been administered a therapeutic cell composition containing CAR expressing cells. In some embodiments, administering the anti-idiotypic antibody to a subject promotes re-expansion of the CAR expressing cells in the subject, which, in some cases, may reach or exceed the initial peak level of expansion prior to the administration of the anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody is administered to modulate expansion and/or persistence of the CAR expressing cells at times when the levels of the CAR expressing cells have declined or are not detectable. In some embodiments, CAR expressing cells that re-expanded by the anti-idiotypic antibody exhibit increased potency in a subject to

which it is administered, for example, as compared to the potency prior to administration of the anti-idiotypic antibody.

[0481] In certain embodiments, administration of the anti-idiotypic antibody increases or enhances persistence of the CAR expressing cells in the subject. In some embodiments, the CAR expressing cells are detectable in the subject at or at least 7 days, 14 days, 21 days, 28 days, 35 days, 42 days, 49 days, 56 days, 63 days, 2 months, 3 months, 4 months, 5 months, 6 months, or more than 6 months following the administration of the anti-idiotypic antibody. In some aspects, increased exposure of the subject to the cells includes expansion and/or increased expansion of the cells.

[0482] In some embodiments, the CAR-expressing cells expand in the subject following administration of the anti-idiotypic antibody. In particular embodiments, administering the anti-idiotypic antibody results in a maximum concentration in the blood or serum or other bodily fluid or organ or tissue of the subject, of at least 100, 500, 1000, 1500, 2000, 5000, 10,000 or 15,000 copies of a nucleic acid encoding the CAR per microgram of DNA, or at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 CAR-expressing cells per microliter. In some embodiments, the cells expressing the CAR are detected as at least 10, 20, 30, 40, 50, or 60 % of total PBMCs in the blood of the subject, and/or at such a level for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, 48, or 52 weeks following the administration of the anti-idiotypic antibody or for 1, 2, 3, 4, or 5, or more years following administration of the anti-idiotypic antibody. In some aspects, administering the anti-idiotypic antibody results in at least a 2-fold, at least a 4-fold, at least a 10-fold, or at least a 20-fold increase in copies of nucleic acid encoding the recombinant receptor, *e.g.*, CAR, per microgram of DNA, *e.g.*, in the serum, plasma, blood or tissue, *e.g.*, tumor sample, of the subject. In particular embodiments, administering the anti-idiotypic antibody results in at least a 2-fold, at least a 4-fold, at least a 10-fold, or at least a 20-fold increase in the number of circulating CAR expressing cells in the subject.

[0483] In some aspects, at least about 1×10^2 , at least about 1×10^3 , at least about 1×10^4 , at least about 1×10^5 , or at least about 1×10^6 or at least about 5×10^6 or at least about 1×10^7 or at least about 5×10^7 or at least about 1×10^8 CAR-expressing cells and/or at least 10, 25, 50, 100, 200, 300, 400, or 500, or 1000 CAR expressing cells per microliter, *e.g.*, at least 10 per microliter, are detectable or are present in the subject or fluid, plasma, serum, tissue, or

compartment thereof, such as in the blood, *e.g.*, peripheral blood, or disease site following administration of the anti-idiotypic antibody. In some embodiments, such a number or concentration of cells is detectable in the subject for at least about 20 days, at least about 40 days, or at least about 60 days, or at least about 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 2 or 3 years, following administration of the anti-idiotypic antibody.

[0484] Various delivery systems are known and can be used to administer the anti-idiotypic antibody. In certain embodiments, the anti-idiotypic antibody is administered by encapsulation in and/or attachment to liposomes, microparticles, and microcapsules. Methods of administering the anti-idiotypic antibody include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The anti-idiotypic antibody may be administered by any convenient route, for example by infusion, by bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral, rectal and intestinal mucosa, etc.), and may be administered together with other biologically active agents. Administration can be systemic or local. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. In certain embodiments, the anti-idiotypic antibody is delivered in a vesicle, in particular a liposome (Langer, 1990, *Science* 249:1527-1533), for example a cationic liposome (WO 98140052).

[0485] In some embodiments, there is provided a method of producing a cell composition, comprising introducing into cells a nucleic acid molecule encoding a CAR, thereby generating an input composition, and incubating the input composition with an anti-idiotypic antibody or antigen-binding fragment thereof specific for the antigen-binding domain of the CAR, thereby producing the cell composition. In some embodiments, the CAR comprises a target antibody or antigen-binding fragment thereof that specifically binds to CD19. In some embodiments, the target antibody is antibody SJ25C1 or FMC63 or an antigen-binding fragment thereof. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is an anti-idiotypic antibody or antigen-binding fragment thereof described herein. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is an agonist of the CAR. In some embodiments, the introducing comprises introducing the nucleic acid molecule into the cells by viral transduction, transposition, electroporation, or chemical transfection. In some embodiments, the introducing comprises introducing the nucleic acid molecule in the cells by

transduction with a retroviral vector comprising the nucleic acid molecule, by transduction with a lentiviral vector comprising the nucleic acid molecule, by transposition with a transposon comprising the nucleic acid molecule, or by electroporation or transfection of a vector comprising the nucleic acid molecule.

[0486] In some embodiments, the method further comprises a step of stimulating or activating the cells prior to introducing the nucleic acid molecule encoding the CAR. In some embodiments, activating the cells comprises contacting the cells with an agonist of CD3 and optionally an agonist of CD28. In some embodiments, activating the cells comprising contacting the cells with a reagent comprising agonistic anti-CD3 and anti-CD28 antibodies. In some such embodiments, during at least a portion of the contacting with an anti-CD3/anti-CD28 and/or during at least a portion of introducing the nucleic acid encoding the CAR, the method includes incubating or contacting the cells with the anti-idiotypic antibody or antigen-binding. In some embodiments, the incubation is performed under conditions in which the anti-idiotypic antibody or antigen-binding fragment thereof binds to the CAR, thereby inducing or modulating a signal in one or more cells in the input composition. In some embodiments, the cells comprise T cells.

[0487] In some such embodiments, the T cells comprise CD4+ and/or CD8+ T cells. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a solid support, which optionally comprises or is conjugated to a reagent comprising a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a soluble reagent, which optionally is or comprises a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof. In some embodiments, the reagent comprises a streptavidin mutein. In one exemplary embodiment, the anti-idiotypic antibody comprises a streptavidin-binding peptide or other streptavidin binding moiety capable of binding to a streptavidin or streptavidin mutein molecule present on or immobilized on the soluble reagent, which, in some cases, can be dissociated in the presence of a competition substance, such as biotin. Exemplary of such systems include those described in PCT published patent application No.

WO2015/158868. In some embodiments, the incubation is for at least or about at least 5 minutes, 10 minutes, 30 minutes, 60 minutes, 2 hours, 6 hours, 12 hours, 24 hours, 36, 48 hours,

72 hours or 96 hours. In some embodiments, the input composition comprises less than or less than about 60%, less than or less than about 50%, less than or less than about 40%, less than or less than about 30%, less than or less than about 20% or less than or less than about 10% CAR-expressing cells as a percentage of the total cells in the composition. In some embodiments, the number of CAR-expressing cells in the output composition is increased by greater than 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold or more compared to the number of CAR-expressing cells in the input composition; and/or the percentage of CAR-expressing in the output composition compared to the total cells in the composition is increased by greater than 10 %, 20 %, 40 %, 50 %, 60 %, 70 %, 80 % or more. In some embodiments, prior to incubating, the cells are not selected or enriched for CAR-expressing cells. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0488] In some embodiments, there is provided a method of monitoring activity of a CAR comprising a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, including the steps of incubating a sample comprising T cells transduced with the CAR with an agonistic anti-idiotypic antibody or antigen-binding fragment thereof that targets or binds the CAR; and/or determining the presence, absence or amount of activation, stimulation and/or expansion of the CAR T cells, thereby monitoring the activity of the CAR-T cells. In some embodiments, such methods can be used for validating the CAR, in which case the method can include c) validating the CAR based on the level of activation, stimulation and/or expansion of CAR-T cells.

[0489] In some embodiments, activation, stimulation and/or expansion of CAR T cells is assessed by determining the viability, proliferation, and/or expression of T cell activation markers in the CAR T cells following a period of incubation with the anti-idiotypic antibody. In some embodiments, viability of CAR T cells is assessed by calculating the percent of living versus total T cells transduced with the CAR following incubation with the anti-idiotypic antibody. In some embodiments, proliferation of CAR T cells is assessed by dye dilution of a dye used to stain the CAR T cells prior to incubation with the anti-idiotypic antibody. In some

embodiments, expression of T cell activation markers is assessed by flow cytometry with staining for antibodies recognizing the T cell activation markers. In some embodiments, the T cell activation markers are selected from the group consisting of CD25, CD26, CD27, CD28, CD30, CD69, CD71, CD134, CD137, and CD154. In some embodiments, the period of incubation is from about 1 to about 10 days (such as about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days, including any ranges between these values). In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0490] In some embodiments, there is provided a method of monitoring a preparation of CAR T cells, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising a) incubating a portion of the preparation with an agonistic anti-idiotypic antibody or antigen-binding fragment thereof that targets or binds the CAR; and b) determining the presence, absence or amount of activation, stimulation and/or expansion of the CAR T cells. In some embodiments, the preparation of CAR-T cells can be cells produced or manufactured under particular conditions desirable to be tested. In some embodiments, the monitoring is carried out in connection with a release assay, such as for validating the cells prior to administration to a subject. In some aspects, the method further includes c) validating the preparation based on the level of activation of the CAR T cells. In some embodiments, activation of CAR T cells in the preparation is assessed by determining the viability, proliferation, and/or expression of T cell activation markers in the CAR T cells following a period of incubation with the anti-idiotypic antibody. In some embodiments, viability of CAR T cells is assessed by calculating the percent of living versus total T cells transduced with the CAR following incubation with the anti-idiotypic antibody. In some embodiments, proliferation of CAR T cells is assessed by dye dilution of a dye used to stain the CAR T cells prior to incubation with the anti-idiotypic antibody. In some embodiments, expression of T cell activation markers is assessed by flow cytometry with staining for antibodies recognizing the T cell activation markers. In some embodiments, the T cell activation markers are selected from the group consisting of CD25, CD26, CD27, CD28, CD30, CD69,

CD71, CD134, CD137, and CD154. In some embodiments, the period of incubation is from about 1 to about 10 days (such as about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days, including any ranges between these values). In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

C. Use in Cell Inactivation/Depletion

[0491] In some embodiments, the provided anti-idiotypic antibodies or antigen-binding fragments thereof are antagonists and/or exhibit specific activity to inhibit, ablate, and/or deplete (for example, kill via antibody-dependent cell-mediated cytotoxicity, ADCC) cells expressing a target antibody, such as an anti-CD19 antibody (*e.g.*, antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof. Also provided are methods involving use of the provided anti-idiotypic antibodies, and molecules (such as conjugates and complexes) containing one or more of such anti-idiotypic antibodies, for inactivation, ablation, and/or depletion of CAR T cells, wherein the CAR comprises a target antibody, such as an anti-CD19 antibody (*e.g.*, antibody SJ25C1 or FMC63), or an antigen-binding fragment thereof.

[0492] The methods in some embodiments include treating, contacting, and/or incubating a composition and/or a sample comprising T cells transduced with a CAR with the anti-idiotypic antibody. In certain embodiments, the methods further include detecting whether the CAR T cells are inactivated, such as by assessing the viability, proliferation, and/or expression of activation markers in the CAR T cells. In some embodiments, the methods are in association with a therapy comprising administration of CAR T cells. The methods in some embodiments include administering the anti-idiotypic antibody to an individual. In one embodiment, an anti-idiotypic antibody or conjugate is used to ablate and/or deplete (such as kill) CAR T cells in an individual. In some embodiments, the target antibody is an anti-CD19 antibody. In some embodiments, the target antibody is or is derived from antibody SJ25C1 or FMC63 or an antigen-binding fragment thereof. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target

antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0493] In some embodiments, the anti-idiotypic antibody is administered to deplete, reduce, and/or decrease the number of CAR expressing cells in a subject. In particular embodiments, administration of the anti-idiotypic antibody depletes, reduces, and/or decreases the amount of CAR expressing cells, e.g., circulating CAR-T cells, by at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.9%, 100% or about 100%. In certain embodiments, the depletion, reduction, and/or decrease is in relation to an amount of CAR expressing cells in the subject prior to the administration of the anti-idiotypic antibody. In particular embodiments, the depletion, reduction, and/or decrease is in relation to an amount of CAR expressing cells in a subject that is not administered the anti-idiotypic antibody. In some embodiments, CAR expressing cells are not detectable in the subject following administration of the anti-idiotypic antibody. In particular embodiments, the anti-idiotypic antibody is a human or humanized antibody.

[0494] In some embodiments, there is provided a method of inactivating CAR T cells, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising incubating a sample comprising the CAR T cells with an antagonistic anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR, thereby inactivating the CAR T cells in the sample. In some embodiments, the anti-idiotypic antibody is used in an amount sufficient to attenuate the activation of the CAR T cells in the sample. In some embodiments, the anti-idiotypic antibody is used in an amount sufficient to substantially inactivate the CAR T cells in the sample. In some embodiments, incubation with the anti-idiotypic antibody results in ablation and/or depletion of CAR T cells in the sample. In some embodiments, the anti-idiotypic antibody is used in an amount sufficient to result in clearance of the CAR T cells in the sample. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0495] In some embodiments, the anti-idiotypic antibody is administered to deplete, reduce, and/or decrease the activity of the CAR and/or the CAR expressing cells in a subject. In particular embodiments, administration of the anti-idiotypic antibody reduces and/or decreases stimulation and/or activation of the CAR and/or the CAR expressing cell by at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.9%, 100% or about 100%. In certain embodiments, the reduction and/or decrease is in relation to the stimulation and/or activity of the CAR and/or CAR expressing cells in the subject prior to the administration of the anti-idiotypic antibody. In particular embodiments, the reduction and/or decrease is in relation to stimulation and/or activity of the CAR and/or CAR expressing cells in a subject that is not administered the anti-idiotypic antibody. In some embodiments, the activity and/or stimulation refers to one or more aspects of CAR receptor or CAR T cell activity and may be assessed by any suitable known means, including by any means provided herein. In some embodiments, activity and/or stimulation of the CAR and/or CAR expressing cells are not detectable in the subject following administration of the anti-idiotypic antibody. In particular embodiments, the anti-idiotypic antibody is a human or humanized antibody.

[0496] In some embodiments, the anti-idiotypic antibody is administered to prevent, reduce, and/or decrease the binding and/or the ability of the CAR and/or the CAR expressing cells to bind to the antigen. In particular embodiments, administration of the anti-idiotypic antibody reduces and/or decreases antigen binding of the CAR and/or the CAR expressing cell by at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.9%, 100% or about 100%. In certain embodiments, the reduction and/or decrease is in relation to the antigen binding and/or the ability of the CAR and/or CAR expressing cells to bind to the antigen in the subject prior to the administration of the anti-idiotypic antibody. In particular embodiments, the reduction, and/or decrease is in relation to antigen binding and/or the ability to bind the antigen of the CAR and/or CAR expressing cells in a subject that is not administered the anti-idiotypic antibody. In particular embodiments, the anti-idiotypic antibody is a human or humanized antibody.

[0497] In some embodiments, there is provided a method of ablating and/or depleting (such as killing) CAR T cells, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising incubating a sample comprising the CAR T cells with an anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR, thereby ablating and/or depleting CAR T cells in the sample. In some embodiments, the ablating and/or depleting is by antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, the anti-idiotypic antibody is used in an amount sufficient to result in ablation and/or depletion of substantially all of the CAR T cells in the sample. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0498] In some embodiments, there is provided a method of adjusting a CAR T cell therapy in an individual, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising administering an antagonistic anti-idiotypic antibody or antigen-binding fragment thereof targeting the CAR to the individual, thereby inactivating the CAR T cells. In some embodiments, the anti-idiotypic antibody is administered in an amount sufficient to attenuate the activation of the CAR T cells in the individual. In some embodiments, the anti-idiotypic antibody is administered in an amount sufficient to substantially inactivate the CAR T cells in the individual. In some embodiments, administration of the anti-idiotypic antibody results in ablation and/or depletion of CAR T cells in the individual. In some embodiments, the anti-idiotypic antibody is administered in an amount sufficient to result in clearance of the CAR T cells in the individual. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

[0499] In some embodiments, there is provided a method of adjusting a CAR T cell therapy in an individual, wherein the CAR comprises a target antibody, such as antibody SJ25C1 or FMC63, or an antigen-binding fragment thereof, comprising administering an anti-idiotypic antibody immunoconjugate targeting the CAR to the individual, wherein the anti-idiotypic antibody immunoconjugate comprises a cytotoxic agent. In some embodiments, the anti-idiotypic antibody immunoconjugate is administered in an amount sufficient to attenuate the CAR T cell therapy in the individual. In some embodiments, the anti-idiotypic antibody immunoconjugate is administered in an amount sufficient to substantially stop the CAR T cell therapy in the individual. In some embodiments, the anti-idiotypic antibody immunoconjugate is administered in an amount sufficient to result in clearance of the CAR T cells in the individual. In some embodiments, the cytotoxic agent is selected from the group consisting of chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), and radioactive isotopes. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24. In some embodiments, the target antibody or antigen-binding fragment thereof comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

D. Use in Binding Assay or Method

[0500] Provided herein are methods for assessing the presence or absence of a molecule in a sample that binds to a chimeric antigen receptor (CAR), such as the extracellular domain of a CAR or to a portion thereof containing the antigen-binding domain. In some embodiments, the methods can be used to assess the presence or absence of a humoral response or antibody response in a subject to an administered cell therapy comprising a chimeric antigen receptor (CAR). In some embodiments, the chimeric antigen receptor comprises a target antibody that is antibody FMC63 or an antigen-binding fragment thereof. In some embodiments, the chimeric antigen receptor comprises a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof. In some embodiments, an anti-idiotypic antibody or antigen-binding fragment thereof specific to the extracellular domain of the CAR, such as any described herein, can be used as a positive control in the method.

[0501] In particular embodiments, the method includes contacting a sample with an anti-idiotypic antibody or antigen-binding fragment thereof specific to the extracellular domain of the CAR at a concentration of between 10 ng/ml and 100 µg/ml, between 100 ng/ml and 1.0 µg/ml, between 250 ng/ml and 10 µg/ml, between 250 ng/ml and 1 µg/ml, between 1 µg/ml and 10 µg/ml, between 250 ng and 2.5 µg/ml, or between 1 µg/ml and 10 µg/ml of the anti-idiotypic antibody. In some embodiments, the concentration of the anti-idiotypic antibody between 250 ng/ml and 10 µg/ml. In certain embodiments, the concentration of the anti-idiotypic antibody is about 0.1 µg/ml, 0.25 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 1.25 µg/ml, 2 µg/ml, 2.5 µg/ml, or 5 µg/ml of the anti-idiotypic antibody.

[0502] In some aspects, adoptive cell therapy may be associated with development of an immune response in the subject to the cells and/or construct administered. For example, in some cases, exposure to a chimeric receptor may be limited by host immune responses against the recombinant receptors expressed by the administered cells, which may prematurely eliminate the cells. It is observed that even in certain subjects having B cell malignancies, who often are immunocompromised, immune responses can be detected that are specific for regions of receptors expressed by cells administered in adoptive cell therapy. For example, subjects, e.g. human subjects, administered cells genetically engineered with a CAR can develop a specific immune response to an immunogenic region of the chimeric region, including regions that may contain non-human sequences (e.g. murine scFv) and or to a region containing the junction between two domains or portions of the chimeric receptor, e.g. the transmembrane and costimulatory domain of the CAR.

[0503] In some embodiments, there are provided methods that involve contacting or incubating a binding reagent with a sample from a subject having been administered a cell therapy comprising cell engineered with a chimeric antigen receptor in which the binding reagent is a protein that includes the extracellular domain of the CAR or a portion thereof containing the target antibody or the antigen-binding fragment thereof. In some embodiments, the methods further include detecting whether a complex is formed between the binding reagent and a molecule, e.g. binding molecule, such as an antibody, present in the sample, and/or detecting the presence or absence or level of such binding. In certain embodiments, the contacting or incubating is under conditions permissive for binding of the binding reagent to a

molecule present in the sample from the subject. In certain aspects, the method can be further carried out on a positive control sample containing an anti-idiotypic antibody or antigen-binding fragment thereof specific for the CAR, such as any as described. In some embodiments, determining the presence, absence or level of binding of the molecule to the binding reagent can include comparison of the binding or detection to the binding or detection of the positive control sample to the binding reagent.

[0504] In some embodiments, the cell therapy is or comprises genetically engineered cells expressing an anti-CD19 CAR comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof, wherein the binding reagent comprises the extracellular domain of the CAR or a portion thereof comprising the SJ25C1 antibody or the antigen-binding fragment thereof. In some embodiments, the positive control includes an anti-idiotypic antibody as described in subsection I.A.

[0505] In some embodiments, the cell therapy is or comprises genetically engineered cells expressing an anti-CD19 CAR comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof, wherein the binding reagent comprise the extracellular domain of the CAR or a portion thereof comprising the FMC63 antibody or the antigen-binding fragment thereof. In some embodiments, the positive control includes an anti-idiotypic antibody as described in subsection I.B.

[0506] In some embodiments, the methods include detecting whether a complex is formed between the binding reagent and a molecule, e.g. binding molecule, such as an antibody, present in the sample, and/or detecting the presence or absence or level of such binding. In certain embodiments, the contacting or incubating is under conditions permissive for binding of the binding reagent to a molecule present in the sample from the subject. In some aspects, the complex is detected by an immunoassay, optionally a sandwich or bridge assay. For examples, the immunoassay is an enzyme-linked immunosorbent assay (ELISA), chemiluminescent, electrochemiluminescent, surface plasmon resonance (SPR)-based biosensor (e.g., BIAcore), flow cytometry, or Western blot. In some embodiments, the immunoassay is or includes meso scale discovery.

[0507] In some aspects, the immunoassay is a sandwich assay or a bridge assay. In a sandwich or bridge assay, the binding reagent is a first binding reagent and detecting the presence or absence of a molecule or a complex comprising a molecule includes contacting the complex formed between the first binding reagent and molecule with a second binding reagent in which the second binding reagent is an agent that is able to bind to the same or similar molecule as the first binding reagent. In some embodiments, the second binding reagent comprises the extracellular domain of the CAR or a portion thereof. In some aspects, the extracellular domain of the CAR or portion thereof of the first binding agent and the second binding agent is the same or substantially the same.

[0508] In some embodiments, the binding reagent, such as the first and/or second binding reagent, is detectably labeled or is capable of producing a detectable signal. The binding reagent, such as the first and/or second binding reagent, is linked, directly or indirectly, to a detectable label. In some embodiments, the detectable label is or includes a fluorescent label, a chemiluminescent label, an electroluminescent label, a colorimetric label, a bioluminescent label or a radiolabel. In some embodiments, the binding reagent, such as the first and/or second binding reagent is linked, directly or indirectly, to a SULFO-Tag. In some embodiments, at least one of the first and second binding reagent is detectably labeled or is capable of producing a detectable signal and the other of the first and second binding reagent is attached or immobilized to a solid support. In some aspects, the first binding reagent is attached or immobilized to a solid support or capable of being attached or immobilized to a solid support. Methods for directly or indirectly attaching an binding reagent to a solid support are well known in the art. Methods of attachment generally include non-specific adsorption of the binding reagent to the solid support or covalent attachment of the binding reagent, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Methods of attachment also include indirect attachment of the binding reagent to the solid support such as by coating the solid support with a capture reagent, such as streptavidin, and adding affinity labeled binding reagents, such as biotin-labeled reagents, to the solid support so that the interaction between the affinity label (e.g., biotin) and capture reagent (e.g., streptavidin) link the binding reagent to the solid support. In some embodiments, the first binding reagent is linked, directly or indirectly, to a biotin. In some

examples, the first soluble reagent is bound to a solid support coated with streptavidin. In some embodiments, the second binding reagent is linked, directly or indirectly, to a detectably label, optionally a SULFO-Tag.

[0509] In particular embodiments, the sample is contacted with a first binding reagent that is attached, bound, coated, and/or conjugated to a solid surface or support, e.g., a plate or a well. In certain embodiments, the first binding reagent has been attached, bound, coated, and/or conjugated to the solid surface or support by indirect attachment of the binding reagent to the solid support such as by coating the solid support with a capture reagent, such as streptavidin, and adding affinity labeled binding reagents, such as biotin-labeled reagents, to the solid support so that the interaction between the affinity label (e.g., biotin) and capture reagent (e.g., streptavidin) link the binding reagent to the solid support. In some embodiments, the sample is contacted with a second binding reagent that is linked, directly or indirectly, to a SULFO-Tag. In particular embodiments, the first and/or second binding reagent is used at a concentration of between 10 ng/ml and 100 µg/ml, between 100 ng/ml and 1.0 µg/ml, between 250 ng/ml and 10 µg/ml, between 250 ng/ml and 1 µg/ml, between 1 µg/ml and 10 µg/ml, between 250 ng and 2.5 µg/ml, or between 1 µg/ml and 10 µg/ml the anti-idiotypic antibody. In some embodiments, the solid surface or support is incubated with between 250 ng/ml and 10 µg/ml. In certain embodiments, the solid surface or support is incubated with or with about 0.25 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 1.25 µg/ml, 2 µg/ml, 2.5 µg/ml, 5 µg/ml or 10 µg/ml.

[0510] In some embodiments, the sample from a subject having been administered a cell therapy comprising cell engineered with a chimeric antigen receptor is or comprises any bodily fluid sample from the subject. In some aspects, the sample is or comprises whole blood, serum or plasma. In some embodiments, the sample is obtained from the subject within or about within 1 hour to 1 year after initiation of administration of the cell therapy or dose of cells, such as within or about within 6 hours, 12 hours, 24 hours, one week, two weeks, three weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months or twelve months. In some aspects, the sample is obtained from the subject from or from about 1 month to 6 months of initiation of administration of the cell therapy, such as 2 months to 6 months or 2 months to 4 months, for

example, about or approximately 2 months, 3 months, 4 months, 5 months or 6 months after initiation of administration of the cell therapy.

VI. ARTICLES OF MANUFACTURE

[0511] Also provided are articles of manufacture or kits containing the provided anti-idiotypic antibodies and/or compositions. In some embodiments, provided are articles of manufacture comprising an anti-idiotypic antibody or an antigen-binding fragment thereof. In some cases, the anti-idiotypic antibody binds an anti-CD19 antibody or antigen-binding fragment thereof, or a chimeric antigen receptor comprising an anti-CD19 antibody or antigen-binding fragment thereof. In some examples, the anti-CD19 antibody is SJ25C1 or FMC63. In some aspects, a conjugate containing the anti-idiotypic antibodies described herein are provided in the articles of manufacture or kits.

[0512] In some embodiments, the kit or article of manufacture includes the anti-idiotypic antibody or antigen binding fragment thereof and a binding reagent containing the extracellular domain, or portion of an extracellular domain, of a chimeric antigen receptor (CAR) to which the anti-idiotypic antibody binds, such as specifically binds. In some embodiments, the extracellular domain of the CAR is or includes the anti-CD19 antibody (e.g., FMC63 or SJ25C1) or an antigen-binding fragment thereof.

[0513] In some embodiments, the binding reagent is a first binding reagent and the kit or article of manufacture additionally includes a second binding reagent. In such examples, the second binding reagent is an agent that is able to bind to the same or similar molecule as the first binding reagent. In some embodiments, the second binding reagent comprises the extracellular domain of the CAR or a portion thereof. In some aspects, the extracellular domain of the CAR or portion thereof of the first binding agent and the second binding agent is the same or substantially the same.

[0514] In some embodiments, the binding reagent, or at least one of the first and second binding reagents, is attached to a label (e.g. detectable label) such as a label described herein. In some embodiments, at least one of the first and second binding reagent is attached to a solid support or capable of being attached to a solid support, such as a solid support described herein. In some aspects, one of the first and second binding reagent is detectably labeled or is capable of

producing a detectable signal and the other of the first and second binding reagent is attached or immobilized to the solid support. In some embodiments, the binding reagents are provided as a kit or as part of a system as described elsewhere herein for use in connection with an immunoassay (e.g. sandwich or bridge assay). In some embodiments, the first binding reagent is bound to a solid support, optionally a streptavidin coated solid support. In some embodiments, the second soluble protein is linked directly or indirectly to a detectable label, such as SULFO-Tag.

[0515] In some embodiments, the kit further comprises an anti-idiotypic antibody or antigen-binding fragment. In some aspects, the anti-idiotypic antibody binds an anti-CD19 antibody or antigen-binding fragment thereof, or a chimeric antigen receptor comprising an anti-CD19 antibody or antigen-binding fragment thereof. In some examples, the anti-CD19 antibody is SJ25C1 or FMC63. In some embodiments, the anti-idiotypic antibody or antigen-binding fragment thereof is provided as a positive control sample. In some examples, the positive control sample forms a complex with the first and second soluble proteins or reagents which contains regions of the extracellular domain of a chimeric antigen receptor (CAR) comprising the CD19 antibody or an antigen-binding fragment thereof.

[0516] In some embodiments, the kit or article of manufacture comprises reagents or components for carrying out any of the provided methods. In some embodiments, the article of manufacture or kit comprises one or more reagent or other materials desirable from a commercial, therapeutic, and user standpoint including secondary antibodies, affinity labels, capture reagents, buffers, diluents, signal detection agents, filters, needles, syringes, capillary tubes, and package inserts with instructions for use.

[0517] In some embodiments, the kits can be provided as articles of manufacture that include packing materials for the packaging of the antibodies or compositions thereof or the one or more additional reagents, e.g. binding reagents, or components. For example, the kits can contain containers, bottles, tubes, vial and any packaging material suitable for separating or organizing the components of the kit.

[0518] In some embodiments, the kit includes one or more containers. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The one or more containers may be formed from a variety of

materials such as glass or plastic. The one or more containers hold a composition comprising an antibody or other reagents, e.g. binding reagents, for use in the methods. The article of manufacture or kit herein may comprise the antibodies or reagents in separate containers or in the same container. In some embodiments, the one or more containers holding the composition may be a single-use vial or a multi-use vial, which, in some cases, may allow for repeat use of the reconstituted composition.

[0519] In some embodiments, the article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, therapeutic agents and/or package inserts with instructions for use.

[0520] The articles of manufacture may include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container in some embodiments holds a composition containing an anti-idiotypic antibody as provided herein which is by itself or is combined with another composition effective for treating, preventing and/or diagnosing a disease or condition. In some embodiments, the container has a sterile access port. Exemplary containers include an intravenous solution bags, vials, including those with stoppers pierceable by a needle for injection. The article of manufacture may include a first container with a composition contained therein, wherein the composition includes the anti-idiotypic antibody. Alternatively, or additionally, the article of manufacture may further include another or the same container comprising an acceptable buffer. It may further include other materials such as other buffers, diluents, filters, needles, and/or syringes.

[0521] In some embodiments, the article of manufacture or kit comprises a solid support, including a solid support formed of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol, nitrocellulose, cellulose, nylon, silicones and other material well known in the art that is used in a solid support for direct or indirect attachment of a binding reagent as described. Solid supports included in the articles of manufacture or kits provided herein include, but are not limited to, a bead, column (e.g.,

chromatography column, etc.), an array (e.g., microarray, nanoarray, etc.), an assay plate, a cartridge, a stick, a filter, a strip or any other solid support described herein.

[0522] In some embodiments, the article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, therapeutic agents and/or package inserts with instructions for use.

[0523] In some embodiments, the kit can, optionally, include instructions. Instructions typically include a tangible expression describing the antibodies and, optionally, other components included in the kit, e.g. binding reagent, and methods for using the antibodies and/or other components in or in conjunction with any of the uses or methods as described. In some embodiments, the instructions are provided as a label or a package insert, which is on or associated with the container. In some embodiments, the instructions may indicate directions for reconstitution and/or use of the composition.

[0524] In some embodiments, instructions are provided for using the anti-idiotypic antibody to detect an SJ25C1 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof, such as in accord with or conjunction with any of the methods or assays as described. In some examples, instructions are provided for using the anti-idiotypic antibody to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody SJ25C1 or an antigen-binding fragment thereof. In some examples, instructions are provided for using the anti-idiotypic antibody to stimulate an input composition comprising cells expressing a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof.

[0525] In some embodiments, instructions are provided for using the anti-idiotypic antibody to detect an FMC63 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the FMC63 antibody or antigen-binding fragment thereof, such as in accord with or conjunction with any of the methods or assays as described. In some aspects, instructions are provided for using the anti-idiotypic antibody to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody FMC63 or an antigen-binding fragment thereof. In some embodiments, instructions are provided for

using the anti-idiotypic antibody to stimulate an input composition comprising cells expressing a chimeric antigen receptor comprising the FMC63 antibody or antigen-binding fragment thereof.

[0526] In some embodiments, instructions are provided for use of the kit provided to detect a molecule that binds to a chimeric antigen receptor of the cell therapy, such as an antibody, e.g. an antibody produced by a humoral immune response to the chimeric antigen receptor (CAR). In some embodiments, the instructions are provided for contacting a binding reagent with a sample from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the anti-CD19 antibody (e.g., FMC63 or SJ25C1) or an antigen-binding fragment thereof, wherein the binding reagent comprises the extracellular domain of the CAR or a portion of the extracellular domain comprising the target antibody or the antigen-binding fragment thereof. In some aspects, the instructions also specify detecting the presence or absence of a complex comprising the binding reagents and a molecule from the sample that binds to both the first and the second binding reagent, optionally wherein the molecule is or comprises an antibody. In some aspects, the CD19 antibody is SJ25C1 or FMC63. In some further embodiments, instructions for using the binding reagents and the positive control sample are provided.

VII. DEFINITIONS

[0527] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0528] As used herein, reference to a “corresponding form” of an antibody means that when comparing a property or activity of two antibodies, the property is compared using the same form of the antibody. For example, if it is stated that an antibody has greater activity compared to the activity of the corresponding form of a first antibody, that means that a particular form, such as a scFv of that antibody, has greater activity compared to the scFv form of the first antibody.

[0529] As used herein, recitation that nucleotides or amino acid positions "correspond to" nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence listing, refers to nucleotides or amino acid positions identified upon alignment with the disclosed sequence to maximize identity using a standard alignment algorithm, such as the GAP algorithm. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides. In general, to identify corresponding positions, the sequences of amino acids are aligned so that the highest order match is obtained (see, e.g. : Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carrillo et al. (1988) SIAM J Applied Math 48: 1073).

[0530] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0531] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0532] The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0533] An “isolated” antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848: 79-87 (2007).

[0534] An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0535] “Isolated nucleic acid encoding an anti-idiotypic antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0536] The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0537] As used herein, “percent (%) amino acid sequence identity” and “percent identity” when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence (e.g., the subject antibody or fragment) that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent

sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0538] An amino acid substitution may include replacement of one amino acid in a polypeptide with another amino acid. The substitution may be a conservative amino acid substitution or a non-conservative amino acid substitution. Amino acid substitutions may be introduced into a binding molecule, e.g., antibody, of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0539] Amino acids generally can be grouped according to the following common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

[0540] In some embodiments, conservative substitutions can involve the exchange of a member of one of these classes for another member of the same class. In some embodiments, non-conservative amino acid substitutions can involve exchanging a member of one of these classes for another class.

[0541] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of

nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0542] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0543] As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. For example, “a” or “an” means “at least one” or “one or more.” It is understood that aspects and variations described herein include “consisting” and/or “consisting essentially of” aspects and variations.

[0544] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0545] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”.

[0546] The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Polypeptides, including the provided antibodies and antibody chains and other peptides, e.g., linkers, may include amino

acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0547] As used herein, a composition refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0548] As used herein, a statement that a cell or population of cells is “positive” for a particular marker refers to the detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0549] As used herein, a statement that a cell or population of cells is “negative” for a particular marker refers to the absence of substantial detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

VIII. EXEMPLARY EMBODIMENTS

[0550] Among the embodiments provided herein are:

1. An anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof.
2. The anti-idiotypic antibody or antigen-binding fragment of embodiment 1, wherein the antibody or antigen-binding fragment comprises:
 - a light chain variable (VL) region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 5; and/or
 - a heavy chain variable (VH) region comprising at least 90% sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 1.
3. An antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment comprises:
 - a VL region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 5; and/or
 - a VH region comprising at least 90 % sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 1.
4. The anti-idiotypic antibody or antigen-binding fragment of embodiment 2 or embodiment 3, wherein:
 - the VH region comprises a heavy chain complementarity determining region 3 (CDR-H3) comprising the amino acid sequence set forth in SEQ ID NO: 11 or 84 or comprising a CDR-H3 contained within the VH sequence set forth in SEQ ID NO: 1; and/or
 - the VL region comprises a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence set forth in SEQ ID NO: 14 or 87 or comprising a CDR-L3 contained within the VL sequence set forth in SEQ ID NO: 5.
5. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 2-4, wherein:
 - the VH region comprises a CDR-H1 and a CDR-H2, respectively, comprising the amino acid sequences of CDR-H1 and CDR-H2 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 1; and/or

the VL region comprises a CDR-L1 and CDR-L2, respectively, comprising the amino acid sequences of CDR-L1 and CDR-L2 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 5.

6. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 2-5, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 9, 78, 79, or 80, a CDR-H2 set forth in SEQ ID NO: 10, 81, 82, or 83, and a CDR-H3 set forth in SEQ ID NO: 11 or 84; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 12 or 85, a CDR-L2 set forth in SEQ ID NO: 13 or 86, and a CDR-L3 set forth in SEQ ID NO: 14 or 87.

7. An anti-idiotypic antibody or antigen-binding fragment thereof comprising: a CDR-H1, a CDR-H2, and a CDR-H3, respectively, comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 1; and/or

a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 5.

8. An anti-idiotypic antibody or antigen-binding fragment thereof comprising: a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 9, 78, 79, or 80, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 10, 81, 82 or 83, and a CDR-H3 comprising the amino acid sequence set forth as SEQ ID NO: 11 or 84; and/or

a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 12 or 85, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 13 or 86, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 14 or 87.

9. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-8, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 1; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 5.

10. The anti-idiotypic antibody or antigen-binding fragment thereof of embodiment 9, wherein the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 1 and the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 5.

11. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-10, wherein the target antibody or antigen-binding fragment comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

12. An anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof.

13. The anti-idiotypic antibody or antigen-binding fragment of embodiment 12, wherein the antibody or antigen-binding fragment comprises:

a light chain variable (VL) region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62; and/or

a heavy chain variable (VH) region comprising at least 90% sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58.

14. An antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment comprises:

a VL region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62; and/or

a VH region comprising at least 90 % sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58.

15. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-14, wherein:

the VH region comprises:

a heavy chain complementarity determining region 1 (CDR-H1) comprising the amino acid sequence of GYX₃FX₅X₆YX₈MX₁₀ (SEQ ID NO: 108), wherein X₃ is T or S, X₅ is T or S, X₆ is D or R, X₈ is Y or W, and X₁₀ is K or N;

a heavy chain complementarity determining region 2 (CDR-H2) comprising the amino acid sequence WIGX₄IX₆PX₈X₉X₁₀X₁₁TX₁₃X₁₄NQX₁₇FKX₂₀ (SEQ ID NO: 109),

wherein X₄ is D or M, X₆ is N or H, X₈ is N or S, X₉ is N or D, X₁₀ is G or S, X₁₁ is G or E, X₁₃ is D or R, X₁₄ is Y or L, X₁₇ is N or K, and X₂₀ is G or D;

a heavy chain complementarity determining region 3 (CDR-H3) comprising the amino acid sequence AX₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅ (SEQ ID NO: 110), wherein X₂ is R or S, X₃ is E or I, X₄ is G or Y, X₅ is N or Y, X₆ is N or E, X₇ is Y or null, X₈ is G or null, X₉ is S or null, X₁₀ is R or null, X₁₁ is D or null, X₁₂ is A or null, X₁₃ is M or null, X₁₄ is D or E, and X₁₅ is Y or A; and/or

the VL region comprises:

a light chain complementarity determining region 3 (CDR-L1) comprising the amino acid sequence X₁AX₃X₄X₅X₆X₇X₈YX₁₀X₁₁WY (SEQ ID NO: 111), wherein X₁ is S or R, X₃ is S or R, X₄ is S or G, X₅ is G or N, X₆ is V or I, X₇ is I or H, X₈ is N or null, X₁₀ is M or L, and X₁₁ is Y or A;

a light chain complementarity determining region 2 (CDR-L2) comprising the amino acid sequence X₁X₂X₃YX₅X₆X₇X₈LAX₁₁ (SEQ ID NO: 112), wherein X₁ is P or L, X₂ is W or L, X₃ is I or V, X₅ is L or N, X₆ is T or A, X₇ is S or K, X₈ is N or T, and X₁₁ is S or D;

a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence QX₂X₃X₄X₅X₆PX₈T (SEQ ID NO: 113), wherein X₂ is Q or H, X₃ is W or F, X₄ is S or W, X₅ is S or W, X₆ is N or T, and X₈ is L or Y.

16. The anti-idiotypic antibody or antigen-binding fragment of embodiment 15, wherein:

the complementarity determining region 3 (CDR-H3) comprises the amino acid sequence set forth in SEQ ID NO: 94 or 104 or comprises a CDR-H3 contained within the VH sequence set forth in SEQ ID NO: 36 or 58; and/or

the light chain complementarity determining region 3 (CDR-L3) comprises the amino acid sequence set forth in SEQ ID NO: 97 or 107 or comprising a CDR-L3 contained within the VL sequence set forth in SEQ ID NO: 40 or 62.

17. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-16, wherein:

the VH region comprises a CDR-H1 and a CDR-H2, respectively, comprising the amino acid sequences of CDR-H1 and CDR-H2 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or

the VL region comprises a CDR-L1 and CDR-L2, respectively, comprising the amino acid sequences of CDR-L1 and CDR-L2 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62.

18. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-17, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 88, 89, 90, 98, 99, or 100, a CDR-H2 set forth in SEQ ID NO: 91, 92, 93, 101, 102, or 103 and a CDR-H3 set forth in SEQ ID NO: 94 or 104; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 95 or 105, a CDR-L2 set forth in SEQ ID NO: 96 or 106, and a CDR-L3 set forth in SEQ ID NO: 97 or 107.

19. An anti-idiotypic antibody or antigen-binding fragment thereof comprising:
a CDR-H1, a CDR-H2, and a CDR-H3, respectively, comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or

a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62.

20. An anti-idiotypic antibody or antigen-binding fragment thereof comprising:
a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 88, 89, 90, 98, 99, or 100, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 91, 92, 93, 101, 102, or 103, and a CDR-H3 comprising the amino acid sequence set forth as SEQ ID NO: 94 or 104; and/or

a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 95 or 105, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 96 or 106, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 97 or 107.

21. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 13-21, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36 or 58; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40 or 62.

22. The anti-idiotypic antibody or antigen-binding fragment thereof of embodiment 21, wherein the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36 or 58 and the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40 or 62.

23. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-22, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 44, 88, 89, or 90, a CDR-H2 set forth in SEQ ID NO: 45, 91, 92, or 93 and a CDR-H3 set forth in SEQ ID NO: 46 or 94; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 47 or 95, a CDR-L2 set forth in SEQ ID NO: 48 or 96, and a CDR-L3 set forth in SEQ ID NO: 49 or 97.

24. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-23, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 65, 98, 99, or 100, a CDR-H2 set forth in SEQ ID NO: 66, 101, 102, or 103 and a CDR-H3 set forth in SEQ ID NO: 67 or 104; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 68 or 105, a CDR-L2 set forth in SEQ ID NO: 69 or 106, and a CDR-L3 set forth in SEQ ID NO: 100 or 107.

25. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-24, wherein:

the VH region comprises a CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36; and/or

the VL region comprises a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40.

26. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-24, wherein:

the VH region comprises a CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 58; and/or

the VL region comprises a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 62.

27. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-25, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40.

28. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 13-24 and 26, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 58; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 62.

29. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-28, wherein the target antibody or antigen-binding fragment is a single chain fragment.

30. The anti-idiotypic antibody or antigen-binding fragment of embodiment 29, wherein the fragment comprises antibody variable regions joined by a flexible linker.

31. The anti-idiotypic antibody or antigen-binding fragment of embodiment 29 or embodiment 30, wherein the fragment comprises an scFv.

32. The anti-idiotypic antibody or antigen-binding fragment of any of embodiments 1-11 and 29-31, wherein the target antibody or antigen-binding fragment:

comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24; and/or

is an scFv comprising the sequence of amino acids set forth in SEQ ID NO: 28.

33. The anti-idiotypic antibody or antigen-binding fragment of any of embodiments 12-31, wherein the target antibody or antigen-binding fragment:

comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31; and/or

is an scFv comprising the sequence of amino acids set forth in SEQ ID NO: 34.

34. An anti-idiotypic antibody or antigen-binding fragment thereof, wherein the anti-idiotypic antibody or antigen-binding fragment specifically binds to the same or an overlapping epitope of a target antibody or antigen-binding fragment thereof as the epitope specifically bound by the anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-33.

35. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-34, wherein:

the target antibody or antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR); and/or

the anti-idiotypic antibody or antigen-binding fragment specifically binds the target antibody or antigen-binding fragment comprised within or included in the antigen-binding domain of the extracellular portion of a CAR.

36. The anti-idiotypic antibody or antigen-binding fragment of embodiment 35, wherein the target antibody or antigen-binding fragment is an scFv and the anti-idiotypic antibody or antigen-binding fragment specifically binds to an epitope in the scFv of the CAR.

37. The anti-idiotypic antibody or antigen-binding fragment thereof of any of embodiments 1-11, 29-32 and 35, wherein the antibody or fragment specifically binds to a single chain variable fragment (scFv) derived from antibody SJ25C1 comprised in the extracellular portion of a chimeric antigen receptor, optionally wherein the scFv derived from antibody SJ25C1 comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24; and/or comprises the sequence of amino acids set forth in SEQ ID NO: 28.

38. The anti-idiotypic antibody or antigen-binding fragment thereof of any of embodiments 12-31 and 33-35, wherein the antibody or fragment specifically binds to a single chain variable fragment (scFv) derived from antibody FMC63 comprised in the extracellular portion of a chimeric antigen receptor, optionally wherein the scFv derived from antibody FMC63 comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31; and/or comprises the sequence of amino acids set forth in SEQ ID NO: 34.39. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-38, wherein the anti-idiotypic antibody or antigen-binding fragment specifically binds to an epitope within or including all or a portion of a complementarity determining region (CDR) of the target antibody or antigen-binding fragment.

40. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 35-39, wherein the CAR further comprises a transmembrane domain linked to the antigen-binding domain via a spacer.

41. The anti-idiotypic antibody of embodiment 40, wherein the spacer comprises an extracellular portion from CD28, which optionally is human CD28.

42. The anti-idiotypic antibody or antigen-binding fragment of embodiment 41, wherein the extracellular portion from CD28 comprises the sequence of amino acids set forth in SEQ ID NO: 27.

43. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 40-42, wherein the transmembrane domain comprises a transmembrane portion of CD28, which optionally is human CD28.

44. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 40-43, wherein the antibody or fragment does not bind to an epitope in the spacer domain of the CAR.

45. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-44, wherein the antibody or fragment does not bind or does not specifically bind to CD28 or a portion thereof, which optionally is human CD28, which optionally comprises an extracellular portion of CD28, which optionally comprises the sequence of amino acids set forth in SEQ ID NO: 27.

46. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-45, wherein the antibody or fragment does not bind to an epitope in an Fc domain, which optionally is a human IgG1 Fc domain.

47. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-46, wherein the target antibody or antigen-binding fragment specifically binds to human CD19.

48. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-47, wherein the anti-idiotypic antibody or fragment does not cross-react with another anti-CD19 antibody, which optionally is comprised in the extracellular antigen-binding domain of another CAR.

49. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-48, wherein the anti-idiotypic antibody or fragment does not cross-react with another CAR.

50. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-49, wherein the anti-idiotypic antibody or fragment is an agonist antibody of a CAR comprising the target antibody or antigen-binding fragment.

51. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-50, wherein the antibody or fragment is an antagonist of a CAR comprising the target antibody or antigen-binding fragment.

52. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-51, which is humanized.

53. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-52, which is recombinant.

54. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-53, which is monoclonal.

55. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-54, which is an antigen-binding fragment.

56. The anti-idiotypic antibody or antigen-binding fragment of embodiment 55, wherein the antigen-binding fragment is selected from among fragment antigen binding (Fab)

fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, a single chain variable fragment (scFv) or a single domain antibody.

57. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-54, comprising at least a portion of an immunoglobulin constant region.

58. The anti-idiotypic antibody or antigen-binding fragment of embodiment 57, wherein the at least a portion of an immunoglobulin constant region comprises an Fc region or a portion of the Fc comprising the CH2 and CH3 domains.

59. The anti-idiotypic antibody or antigen-binding fragment of embodiment 57 or embodiment 58, wherein the constant region is derived from human IgG.

60. The anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 57-59, which is an intact antibody or full-length antibody.

61. A conjugate, comprising the anti-idiotypic antibody or antigen-binding fragment of any one of embodiments 1-60 and a heterologous molecule or moiety.

62. The conjugate of embodiment 61, wherein the heterologous molecule or moiety is a label.

63. The conjugate of embodiment 62, wherein the label is selected from a fluorescent dye, a fluorescent protein, a radioisotope, a chromophore, a metal ion, a gold particle, a silver particle, a magnetic particle, a polypeptide, an enzyme, a streptavidin, a biotin, a luminescent compound or an oligonucleotide.

64. The conjugate of embodiment 62, wherein the heterologous molecule or moiety is a protein, peptide, nucleic acid or small molecule, which optionally is or comprises a toxin, Strep-Tag.

65. A nucleic acid molecule(s) encoding the heavy chain and/or light chain of the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-60.

66. The nucleic acid molecule of embodiment 65, comprising:
a sequence of nucleotides encoding (i) the heavy chain variable region set forth in SEQ ID NO: 15, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 15; or (iii) a degenerate sequence of (i) or (ii); and/or

a sequence of nucleotides encoding (iv) the light chain variable region set forth in SEQ ID NO: 19, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 19; or (vi) a degenerate sequence of (iv) or (v).

67. The nucleic acid molecule of embodiment 65 or embodiment 66, comprising:

a sequence of nucleotides encoding (i) the heavy chain set forth in SEQ ID NO: 17, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 17; or (iii) a degenerate sequence of (i) or (ii); and/or

a sequence of nucleotides encoding (iv) the light chain set forth in SEQ ID NO: 21, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 21; or (vi) a degenerate sequence of (iv) or (v).

68. The nucleic acid molecule of embodiment 65, comprising:

a sequence of nucleotides encoding (i) the heavy chain variable region set forth in SEQ ID NO: 50 or 71, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 50 or 71; or (iii) a degenerate sequence of (i) or (ii); and/or

a sequence of nucleotides encoding (iv) the light chain variable region set forth in SEQ ID NO: 54 or 75, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 54 or 75; or (vi) a degenerate sequence of (iv) or (v).

69. The nucleic acid molecule of embodiment 65 or embodiment 68, comprising:

a sequence of nucleotides encoding (i) the heavy chain set forth in SEQ ID NO: 52 or 73, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 52 or 73; or (iii) a degenerate sequence of (i) or (ii); and/or

a sequence of nucleotides encoding (iv) the light chain set forth in SEQ ID NO: 56 or 76, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 56 or 76; or (vi) a degenerate sequence of (iv) or (v).

70. The nucleic acid molecule of any one of embodiments 65-69, wherein the nucleotide sequence encoding the heavy chain and/or light chain comprises a signal sequence.

71. A vector, comprising the nucleic acid molecule of any one of embodiments 65-70.

72. A cell, comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-41 or the nucleic acid molecule of any one of embodiments 65-70.

73. A method of producing an anti-idiotypic antibody or antigen-binding fragment thereof, comprising expressing the heavy and/or light chain encoded by the nucleic acid molecule of any one of embodiments 65-70 or the vector of embodiment 71 in a suitable host cell and recovering or isolating the antibody.

74. A method of producing an anti-idiotypic antibody or antigen-binding fragment thereof, comprising culturing the cell of embodiment 72 under conditions in which the heavy chain and/or light chain is expressed and recovering or isolating the antibody.

75. An anti-idiotypic antibody or antigen-binding fragment thereof produced by the method of embodiment 73 or embodiment 74.

76. A composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-60, conjugate of any one of embodiments 61-64, or the cell of embodiment 72.

77. The composition of embodiment 76, further comprising a pharmaceutically acceptable excipient.

78. A kit, comprising one or more of the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-60, the conjugate of any one of embodiments 61-64, the nucleic acid of any one of embodiments 65-70, and, optionally, instructions for use.

79. The kit of embodiment 78, further comprising a reagent or support for immobilizing the anti-idiotypic antibody or antigen-binding fragment thereof or conjugate, wherein said reagent or support is a bead, a column, a microwell, a stick, a filter, a strip or a soluble oligomeric streptavidin mutein reagent.

80. A method of detecting a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment.

81. A method of detecting a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and

(b) detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment.

82. The method of embodiment 81, wherein the target antibody or antigen-binding fragment is bound to a cell or expressed on the surface of a cell and detecting in (b) comprises detecting cells bound with the anti-idiotypic antibody.

83. The method of embodiment 82, wherein the cell expresses on its surface a CAR comprising the target antibody or antigen-binding fragment.

84. A method of detecting a CAR comprising a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) detecting cells bound with the anti-idiotypic antibody.

85. A method of detecting a CAR comprising a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36

and 38-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and

(b) detecting cells bound with the anti-idiotypic antibody.

86. The method of any one of embodiments 80-85, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is directly or indirectly labeled for detection.

87. A method of selecting cells from a cell population, comprising:

(a) contacting a cell population expressing a chimeric antigen receptor (CAR) comprising a target antibody or a cell bound to a target antibody with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, wherein the target antibody is the antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) selecting cells bound with the anti-idiotypic antibody.

88. A method of selecting cells from a cell population, comprising:

(a) contacting a cell population expressing a chimeric antigen receptor (CAR) comprising a target antibody or a cell bound to a target antibody with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, wherein the target antibody is the antibody FMC63 or an antigen-binding fragment thereof; and

(b) selecting cells bound with the anti-idiotypic antibody.

89. The method of embodiment 87 or embodiment 88, wherein the cells bound with the anti-idiotypic antibody are selected by affinity-based separation.

90. The method of embodiment 89, wherein the affinity-based separation is immunoaffinity-based separation.

91. The method of embodiment 89 or embodiment 90, wherein the affinity-based separation is by flow cytometry.

92. The method of embodiment 89 or embodiment 90, wherein the affinity-based separation is by magnetic activated cell sorting. 93. The method of embodiment 89 or embodiment 90, wherein the affinity-based separation comprises affinity chromatography. 94.

The method of embodiment 92 or embodiment 93, wherein the anti-idiotypic antibody is reversibly bound or immobilized to a support or a stationary phase.

95. A method of stimulating cells, comprising incubating an input composition comprising cells expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, thereby generating an output composition comprising stimulated cells.

96. A method of stimulating cells, comprising incubating an input composition comprising cells expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, thereby generating an output composition comprising stimulated cells.

97. A method of producing a cell composition, comprising:

(a) introducing into cells a nucleic acid molecule encoding a chimeric antigen receptor (CAR), thereby generating an input composition; and

(b) incubating the input composition with an anti-idiotypic antibody or antigen-binding fragment thereof specific for the antigen receptor of the CAR, thereby producing the cell composition.

98. The method of embodiment 97, wherein the CAR comprises a target antibody that specifically binds to CD19.

99. The method of embodiment 98, wherein the target antibody is the antibody SJ25C1 or an antigen-binding fragment thereof.

100. The method of any one of embodiments 97-99, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof.

101. The method of embodiment 98, wherein the target antibody is the antibody FMC63 or an antigen-binding fragment thereof.

102. The method of any of embodiments 97-98 and 101, wherein the anti-idiotypic antibody or antigen-binding fragment thereof specifically binds to a target antibody that is antibody FMC63 of any one of embodiments 12-31, 33-36 and 38-60 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof.

103. The method of any one of embodiments 97-102, wherein the introducing in (a) comprises introducing the nucleic acid molecule into the cells by viral transduction, transposition, electroporation, or chemical transfection.

104. The method of any one of embodiments 97-103, wherein the introducing in (a) comprises introducing the nucleic acid molecule in the cells by transduction with a viral vector comprising the nucleic acid molecule, optionally wherein the viral vector is a retroviral vector or a lentiviral vector.

105. The method of any of embodiments 97-103, wherein the introducing in (a) comprises introducing the nucleic acid molecule in the cells by transposition with a transposon comprising the nucleic acid molecule.

106. The method of any of embodiments 97-103, wherein the introducing in (a) comprises introducing the nucleic acid molecule in the cells by electroporation or transfection of a vector comprising the nucleic acid molecule.

107. The method of any one of embodiments 97-106, further comprising a step of activating the cells prior to step (a).

108. The method of embodiment 107, wherein the step of activating the cells comprises contacting the cells with an agonist of CD3 and optionally an agonist of CD28.

109. The method of embodiment 108, wherein the step of activating the cells comprises contacting the cells with a reagent comprising agonistic anti-CD3 and anti-CD28 antibodies.

110. The method of any one of embodiments 95-109, wherein the incubation is performed under conditions in which the anti-idiotypic antibody or antigen-binding fragment thereof binds to the CAR, thereby inducing or modulating a signal in one or more cells in the input composition.

111. The method of any one of embodiments 95-110, wherein the cells comprise T cells.

112. The method of embodiment 111, wherein the T cells comprise CD4+ and/or CD8+ T cells.

113. The method of any one of embodiments 95-112, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a solid support, which optionally comprises or is conjugated to a reagent comprising a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof.

114. The method of any one of embodiments 95-112, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a soluble reagent, which optionally is or comprises a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof.

115. The method of embodiment 113 or embodiment 114, wherein the reagent comprises a streptavidin mutein.

116. The method of any one of embodiments 95-115, wherein the incubation is for at least or about at least 5 minutes, 10 minutes, 30 minutes, 60 minutes, 2 hours, 6 hours, 12 hours, 24 hours, 36, 48 hours, 72 hours or 96 hours.

117. The method of any one of embodiments 95-116, wherein the input composition comprises less than or less than about 60%, less than or less than about 50%, less than or less than about 40%, less than or less than about 30%, less than or less than about 20% or less than or less than about 10% CAR-expressing cells as a percentage of the total cells in the composition.

118. The method of any one of embodiments 95-117, wherein:

the number of CAR-expressing cells in the output composition is increased by greater than 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold or more compared to the number of CAR-expressing cells in the input composition; and/or

the percentage of CAR-expressing in the output composition compared to the total cells in the composition is increased by greater than 10 %, 20 %, 40 %, 50 %, 60 %, 70 %, 80 % or more.

119. The method of any one of embodiments 95-118, wherein prior to the introducing and/or incubating the cells are not selected or enriched for CAR-expressing cells.

120. The method of any one of embodiments 80, 82-84, 86, 87, 89-95, 97-100, 103-119,, wherein the target antibody or antigen-binding fragment comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

121. The method of any one of embodiments 81, 82, 83, 85, 86, 88-94, 96-99, 101 and 102-119, wherein the target antibody or antigen-binding fragment comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

122. A method of purifying an antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) isolating complexes comprising the anti-idiotypic antibody.

123. A method of purifying an antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or the conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and

(b) isolating complexes comprising the anti-idiotypic antibody.

124. The method of embodiment 122 or embodiment 123, wherein the complexes comprising the anti-idiotypic antibody are isolated by affinity-based separation.

125. The method of embodiment 124, wherein the affinity-based separation is immunoaffinity-based separation.

126. The method of embodiment 124, wherein the affinity-based separation is magnetic-based separation.

127. The method of embodiment 124, wherein the affinity-based separation comprises affinity chromatography.

128. A method of identifying an anti-idiotypic antibody or antigen-binding fragment, comprising:

(a) introducing into a subject a soluble immunization reagent comprising an antigen-binding fragment of a target antibody fused to a solubilizing moiety; and

(b) identifying an antibody from the subject that specifically binds to the target antibody or the antigen-binding fragment thereof.

129. The method of embodiment 128, wherein the antigen-binding fragment comprises the variable heavy chain region and/or variable light chain region of the target antibody.

130. The method of embodiments 128 or embodiment 129, wherein the antigen-binding fragment is a single chain fragment.

131. The method of embodiment 130, wherein the antigen-binding fragment is an scFv.

132. The method of any of embodiments 128-131, wherein the antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR).

133. The method of any of embodiments 128-132, wherein the solubilizing moiety is an Fc domain or fragment thereof, which optionally is a human IgG1 Fc.

134. The method of embodiment 133, wherein the solubilizing moiety is an Fc domain lacking the hinge region.

135. The method of embodiment 134, wherein the solubilizing moiety comprises the amino acid sequence set forth in SEQ ID NO: 32.

136. The method of any of embodiments 128-135, wherein identifying the antibody comprises:

(i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas;

(ii) screening the hybridomas for production of antibodies that specifically bind the target antibody or the antigen-binding fragment thereof or a chimeric antigen receptor comprising the antigen-binding fragment; and

(iii) sequencing an antibody from a hybridoma producing an antibody that specifically binds, thereby identifying the anti-idiotypic antibody.

137. The method of any of embodiments 128-136, wherein the target antibody binds to

CD19. 138. The method of any of embodiments 128-137, wherein the antigen-binding fragment of the target antibody is derived from antibody SJ25C1, optionally wherein the antigen-binding fragment of the target antibody comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

139. The method of any of embodiments 128-138, wherein the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody SJ25C1, optionally wherein the scFv comprises the sequence of amino acids set forth in SEQ ID NO:28.

140. The method of any of embodiments 128-137, wherein the antigen-binding fragment of the target antibody is derived from antibody FMC63, optionally wherein the antigen-binding fragment of the target antibody comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

141. The method of any of embodiments 128-137 and 140, wherein the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody FMC63, optionally wherein the scFv comprises the sequence of amino acids set forth in SEQ ID NO: 34.

142. A method of depleting cells, comprising administering, to a subject, a composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof.

143. A method of depleting cells, comprising administering, to a subject, a composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or conjugate of any one of embodiments 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof.

144. The method of embodiment 109, wherein the depletion occurs via antibody-dependent cell-mediated cytotoxicity (ADCC).

145. A method of determining the presence or absence of a molecule that binds to a chimeric antigen receptor (CAR), the method comprising:

(a) contacting a binding reagent with a sample from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof under conditions to form a complex comprising the binding reagent and a molecule from the sample that binds to the binding reagent, wherein the binding reagent comprise the extracellular domain of the CAR or a portion thereof comprising the target antibody or the antigen-binding fragment thereof; and

(b) detecting the presence or absence of the complex, thereby determining the presence or absence of a molecule that binds the CAR.

146. The method of embodiment 145, further comprising carrying out steps (a) and (b) on a positive control sample and, optionally, determining the presence or absence of the molecule by comparison to the positive control, wherein the positive control sample comprises the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of embodiments 61-64 that specifically binds to the target antibody or an antigen-binding fragment thereof.

147. A method of determining the presence or absence of a molecule that binds to a chimeric antigen receptor (CAR), the method comprising:

(a) contacting a binding reagent with a sample from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof under conditions to form a complex

comprising the binding reagent and a molecule from the sample that binds to the binding reagent, wherein the binding reagent comprises the extracellular domain of the CAR or a portion of the extracellular domain comprising the target antibody or the antigen-binding fragment thereof;

(b) detecting the presence or absence of the complex.

148. The method of embodiment 147, further comprising carrying out steps (a) and (b) on a positive control sample and, optionally, determining the presence or absence of the molecule by comparison to the positive control, wherein the positive control sample comprises the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or conjugate of any one of embodiments 61-64 that specifically binds to the target antibody or an antigen-binding fragment thereof.

149. The method of any of embodiments 145-148, wherein the molecule that binds to the binding reagent is or comprises an antibody.

150. The method of any of embodiments 145-149, wherein the binding reagent is detectably labeled or is capable of producing a detectable signal.

151. The method of any of embodiments 145-150, wherein the binding reagent is bound to a solid support or is soluble.

152. The method of any of embodiments 145-153, wherein the complex is detected by an immunoassay.

153. The method of embodiment 152, wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA), chemiluminescent assay, electrochemiluminescent assay, surface plasmon resonance (SPR)-based biosensor (e.g., BIAcore), flow cytometry, or Western blot.

154. The method of embodiment 152 or embodiment 153, wherein the immunoassay comprises meso scale discovery.

155. The method of any of embodiments 152-154, wherein the immunoassay is a sandwich assay or bridge assay.

156. The method of any of embodiments 145-155, wherein the binding reagent is a first binding reagent and detecting the presence or absence of the complex comprises:

(i) contacting the complex formed in step (a) with a second binding reagent, wherein the second binding reagent (1) comprises the extracellular domain of the CAR or a portion thereof comprising the target antibody or the antigen-binding fragment thereof, and (2) is detectably labeled or is capable of producing a detectable signal; and

(ii) assessing the presence or absence of the detectable signal.

157. The method of embodiment 156, wherein:

the first binding reagent is bound to a solid support, optionally wherein first binding reagent is linked, directly or indirectly, to a biotin and/or bound to a solid support through a streptavidin; and/or

the second binding reagent is soluble.

158. The method of embodiment 156 or embodiment 157, wherein the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

159. The method of any of embodiments 150-158, wherein:

the detectable label is or comprises a fluorescent label, a chemiluminescent label, an electroluminescent label, a colorimetric label, a bioluminescent label or a radiolabel; and/or

the detectable signal is or comprises a fluorescent signal, chemiluminescent signal, electroluminescent signal, colorimetric signal, a bioluminescent signal or a radioactive signal.

160. The method of any of embodiments 150-159, wherein the detectable label is or comprises a SULFO-TAG.

161. The method of any of embodiments 145-160, wherein the antigen-binding fragment of the target antibody comprises the variable heavy chain region and/or variable light chain region of the target antibody.

162. The method of embodiment 145-161, wherein the antigen-binding fragment of the target antibody is a single chain fragment.

163. The method of any of embodiments 145-162, wherein the antigen-binding fragment of the target antibody is an scFv.

164. The method of any of embodiments 145-163, wherein the sample comprises whole blood, serum or plasma.

165. An article of manufacture comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 1-11 and 29-32, 34-37 and 39-60 or the

conjugate of any one of embodiments 61-64, and instructions for using the anti-idiotypic antibody to detect an SJ25C1 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof; to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody SJ25C1 or an antigen-binding fragment thereof; to stimulate an input composition comprising cells expressing a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof.

166. An article of manufacture comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of embodiments 12-31, 33-36 and 38-60 or the conjugate of any one of embodiments 61-64, and instructions for using the anti-idiotypic antibody to detect an FMC63 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the FMC63 antibody or antigen-binding fragment thereof; to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody FMC63 or an antigen-binding fragment thereof; to stimulate an input composition comprising cells expressing a chimeric antigen receptor comprising the FMC63 antibody or antigen-binding fragment thereof.

167. An article of manufacture comprising:

a binding reagent comprising the extracellular domain of a chimeric antigen receptor (CAR) comprising a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, said extracellular domain or portion thereof comprising the target antibody or antigen-binding fragment thereof; and

an anti-idiotypic antibody or antigen-binding fragment of any of embodiments 12-31, 33-36 and 38-60 or the conjugate of any one of embodiments 61-64.

168. The article of manufacture of embodiment 167, wherein the binding reagent is a first binding reagent and the article of manufacture further comprises a second binding reagent comprising the extracellular domain or portion thereof of the CAR.

169. The article of manufacture of embodiment 167 or embodiment 168, wherein the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

170. The article of manufacture of any of embodiments 167-169, further comprising instructions for using the binding reagent, optionally the first and second binding reagent, for assaying a sample for the presence or absence of a molecule that binds to the binding reagent using an immunoassay, optionally wherein the immunoassay is a bridge or sandwich immunoassay, optionally wherein the sample is from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof.

171. An article of manufacture comprising:

a binding reagent comprising the extracellular domain of a chimeric antigen receptor (CAR) comprising a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, said extracellular domain or portion thereof comprising the target antibody or antigen-binding fragment thereof; and

an anti-idiotypic antibody or antigen-binding fragment of any of embodiments 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of embodiments 61-64.

172. The article of manufacture of embodiment 171, wherein the binding reagent is a first binding reagent and the article of manufacture further comprises a second binding reagent comprising the extracellular domain or portion thereof of the CAR.

173. The article of manufacture of embodiment 171 or embodiment 172, wherein the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

174. The article of manufacture of any of embodiments 171-173, further comprising instructions for using the binding reagent, optionally the first and second binding reagent, for assaying a sample for the presence or absence of a molecule that binds to the binding reagent using an immunoassay, optionally wherein the immunoassay is a bridge or sandwich immunoassay, optionally wherein the sample is from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof.

175. The article of manufacture of any of embodiments 167-174, wherein the binding reagent, optionally the first and/or second binding reagent, is detectably labeled or capable of producing a detectable signal.

176. The article of manufacture of any of embodiments 168-170 and 172-175, wherein one of the first and second binding reagent is attached to a solid support or is capable of being attached to a solid support and the other of the first and second binding reagent is detectable label or is capable of producing a detectable signal.

177. The method of embodiment 176, wherein the article of manufacture further comprises a solid support, optionally wherein the one of the first and second binding reagent is linked, directly or indirectly to biotin, and the solid support comprises a streptavidin-coated surface.

IX. EXAMPLES

[0551] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1 Generation of anti-idiotypic antibodies against an SJ25C1 variable region-derived antibody

[0552] This example describes the generation of anti-idiotypic antibodies (anti-IDs) recognizing the scFv portion of an exemplary anti-CD19 chimeric antigen receptor (CAR) containing an anti-CD19 scFv with variable heavy and variable light chain regions derived from SJ25C1 (an antibody (in this case an scFv) having a variable region sequence of SEQ ID NO: 23 and 24 separated by a linker set forth in SEQ ID NO: 25), a human CD28-derived extracellular portion, a human CD28-derived transmembrane domain, a human CD28-derived intracellular signaling domain and a human CD3 zeta-derived signaling domain.

A. Hybridoma Generation and Antibody Screening

[0553] Mice were immunized with an extracellular domain (ECD) portion of the CAR (containing an anti-CD19 scFv with variable regions derived from SJ25C1). The ECD portion contained the sequence

[0554] EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQIYPGD
GDTNYNGKFKGQATLTADKSSSTAYMQLSGLTSEDSAVYFCARKTISSVVDYFDYWGQGTTV
TVSSGGGGSGGGGSGGGGSDIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSP

KPLIYSATYRNSGVPDRFTGSGSGTDFTLTITNVQSKDLADYFCQQYNRYPYTSGGGGTKLEIKR (SEQ ID NO: 28) and an extracellular portion from CD28 (SEQ ID NO: 27).

[0555] Serum isolated from immunized mice was tested by ELISA for its ability to bind to the recombinant soluble ECD portion by detection with a secondary antibody. Hybridoma fusion clones were generated and further characterized by ELISA for binding to the ECD and five (5) positive clones were selected. Each selected hybridoma clone was expanded and antibody purified. To permit detection of the antibody in subsequent assays, each antibody was conjugated with Alexa647.

[0556] The antibodies were assessed for the ability to specifically bind to T cells engineered with the anti-CD19 (SJ25C1-derived) CAR, (assessed by comparison with binding to mock-transduced control T cells). T cells were isolated by immunoaffinity-based enrichment from human subjects and cells were activated and transduced with a viral vector encoding the anti-CD19 (SJ25C1-derived) CAR. A series of two fold serial dilutions (starting from 20 µg/mL and diluted to 0.0195 µg/mL) of each of the anti-idiotypic antibodies was individually used to detect surface expression of the CAR by flow cytometry. As a positive control, cells were also assessed for surface expression of the CAR using similar concentrations of a goat-anti-mouse ("GAM") antibody that was able to detect the murine variable region portion of the ECD portion of the CAR. A no antibody control also was tested. The dose-response curve for percent of cells positive for the Alexa647 signal in T cells transduced with the CAR was similar for each of the anti-idiotypic antibodies tested and comparable to that for the positive control GAM antibody, none of which recognized mock T cells transduced with the empty vector. The staining index, which is the difference between the positive and background (mock) peak means and the spread of the background peak, of the anti-idiotypic antibodies and the GAM antibody was also determined and found to be comparable between the anti-idiotypic antibodies and the positive control GAM antibody. Anti-idiotypic antibody clone A-1 (anti-ID A-1) was selected for further characterization.

B. Functional Activity

[0557] Erk1/2 phosphorylation in Jurkat cells engineered to express the CAR described above was assessed by flow cytometry following stimulation with the anti-ID A-1 antibody or an anti-CD3 antibody, in each case in the presence or absence of a cross-linker antibody.

Following incubation, the cells were fixed with formaldehyde, permeabilized, incubated with an antibody specific for phosphorylated Erk1/2 and analyzed by flow cytometry. As shown in FIG. 1, following the incubation of the cells in the presence of the anti-ID A-1 antibody, an increase in Erk1/2 phosphorylation was observed at a level similar to that observed for stimulation with the anti-CD3 antibody in the presence of a crosslinker. Both antibodies were observed to induce similar degrees of Erk1/2 phosphorylation even in the absence of cross-linking.

[0558] Western blotting further was used to compare Erk phosphorylation in CAR-expressing Jurkat cells or parental Jurkat cells not expressing the CAR, following stimulation with anti-ID A-1, an isotype control, or anti-CD3 or in the absence of stimulus, in the presence or absence of a cross-linker antibody. The results indicated that stimulation with the anti-idiotypic antibody specifically increased Erk1/2 phosphorylation in Jurkat cells transduced with the anti-CD19 (SJ25C1-derived) CAR, but not in the parental Jurkat cells (in which only background signal was observed, similar to unstimulated and isotype control-stimulated cells), to a degree similar to that induced by the anti-CD3 antibody. In contrast, the anti-CD3 antibody induced phosphorylation in a non-specific manner, i.e., in both the CAR-expressing and parental Jurkat cell.

C. Sequence Identification

[0559] Sequences of the anti-ID A-1 antibody were determined. Total RNA was extracted from hybridoma cells containing the hybridoma clone expressing anti-ID A-1 and cDNA using isotype-specific anti-sense primers or universal primers using the PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Cat. No. 6110A). RACE PCR was performed to amplify the variable (heavy and light chains) and constant regions of the antibody, which were then separately cloned into a cloning vector and sequenced. Table 2 sets forth the corresponding SEQ ID NOS of the nucleotide or amino acid sequence of the antibody.

Table 2. anti-ID A-1 sequence

	Light Chain			Heavy Chain		
	Full	Variable	Constant	Full	Variable	Constant
Nucleotide	SEQ ID NO: 21	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 15	SEQ ID NO: 16

Amino acid	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID	SEQ ID
	NO: 7	NO: 5	NO: 6	NO: 3	NO: 1	NO: 2

Example 2 Generation of anti-idiotypic antibodies against an FMC63-derived antibody

[0560] This example describes the generation of anti-idiotypic antibodies recognizing the binding domain (scFv) portion of an exemplary anti-CD19 chimeric antigen receptor (CAR) containing an anti-CD19 scFv with VH and VL domains derived from FMC63 (an antibody containing the variable heavy chain (VH) and variable light (VL) light chain sequences set forth in SEQ ID NOs: 30 and 31, respectively). The scFv is set forth in SEQ ID NO:34 and contains the VH and VL regions separated by a linker set forth in SEQ ID NO: 33.

A. Hybridoma Generation and Antibody Screening

[0561] Mice were immunized with a soluble protein containing the scFv portion of this CAR (SEQ ID NO:34), fused to a human IgG1 Fc domain (SEQ ID NO:32; the protein lacked the hinge region). The soluble protein reagent used for immunization is set forth in SEQ ID NO:35.

[0562] Serum isolated from immunized mice was tested by ELISA for ability to bind to the scFv-Fc portion by detection with a secondary antibody. Clones were counter-screened against a peptide containing just the Fc domain (SEQ ID NO: 32), to select for anti-idiotypic antibodies that did not cross react with the Fc portion of the scFv-Fc used to immunize the mice. Clones were also counter-screened against a construct containing an scFv derived from another CD19 antibody, SJ25C1 (variable region sequences of SEQ ID NO: 23 and 24 separated by linker set forth in SEQ ID NO: 25), fused to the hingeless Fc domain (SEQ ID NO: 32) to further select for anti-idiotypic antibodies that did not cross react with a different anti-CD19 antibody.

[0563] Hybridoma fusion clones were generated and 12 candidate clones were further characterized by flow cytometry. Peripheral blood mononuclear cells were isolated from human subjects and cells were activated and transduced with a viral vector encoding the anti-CD19 CAR. For flow cytometry, about 1×10^6 cells per 100 μ L were incubated with 10 μ L of biotin-conjugated anti-idiotypic antibody followed by staining with a PE-conjugated streptavidin. As a positive control for surface expression of the CAR, cells were stained with an anti-EGFR antibody to verify expression of the EGFRt transduction marker, which was a surrogate of CAR expression. As a mock control, PBMCs were transduced with an empty vector that did not

express the CAR. Cells were gated on CD3⁺ CD4⁺/CD8⁺ PBMCs and fluorescence signal was determined. The results showed that the candidate anti-idiotypic antibodies showed specific binding for the CAR on the surface of the PBMCs. To confirm the antibodies were specific for the scFv derived from the anti-CD19 antibody derived from FMC63, similar experiments were performed on cells transduced with an anti-CD19 CAR derived from SJ25C1 as described in Example 1. None of the candidate anti-idiotypic antibodies against a FMC63-derived antibody exhibited specific binding for cells expressing a different anti-CD19 CAR containing an scFv derived from SJ25C1. Anti-idiotypic antibody clones B-1 (anti-ID B-1) and B-2 (anti-ID B-2) were selected for further characterization.

B. Sequence Identification

[0564] Sequences of the anti-ID B-1 and B-2 antibodies were determined. Total RNA was extracted from hybridoma cells containing the hybridoma clones expressing anti-ID B-1 or anti-ID B-2 and cDNA was generated using isotype-specific anti-sense primers or universal primers using the PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Cat. No. 6110A). RACE PCR was performed to amplify the variable (heavy and light chains) and constant regions of the antibodies, which were then separately cloned into a cloning vector and sequenced. Table 3 sets forth the corresponding SEQ ID NOS of the nucleotide or amino acid sequences of the antibodies.

Table 3. anti-ID B-1 and B-2 sequences

	Light Chain			Heavy Chain		
	Full (SEQ ID NO)	Variable (SEQ ID NO)	Constant (SEQ ID NO)	Full (SEQ ID NO)	Variable (SEQ ID NO)	Constant (SEQ ID NO)
Anti-ID B-1 Nucleotide	56	54	55	52	50	51
Anti-ID B-1 Amino acid	42	40	41	38	36	37
Anti-ID B-2 Nucleotide	76	75	55	73	71	72
Anti-ID B-2 Amino acid	63	62	41	60	58	59

Example 3 Effect of plate-bound anti-idiotypic antibody-on T cell stimulation

[0565] This example describes results following the incubation of CAR T cells in the presence of the SJ25C1-specific anti-idiotypic antibody (anti-ID A-1) described in Example 1 or in the presence of the FMC63-derived scFv-specific anti-idiotypic antibody (anti-ID B-1) described in Example 2.

[0566] Peripheral blood mononuclear cells were isolated from human subjects and cells were activated and transduced with a viral vector encoding an anti-CD19 CAR having a binding domain including an scFv with VH and VL domains derived from FMC63 or derived from SJ25C1. Such vectors were introduced into cells to generate T cells engineered to express a FMC63-derived scFv-containing CAR and T cells engineered to express a SJ25C1-derived scFv-containing CAR, respectively. In the case of the FMC63-derived CAR, the CAR-encoding construct further included a sequence that encoded a truncated EGFR (EGFRt), which served as a surrogate marker for transduction and for CAR expression; the EGFRt-coding region was separated from the CAR-encoding sequence by a T2A skip sequence. The engineered cells were assessed in various assays.

[0567] For proliferation studies, engineered T cells were labeled with 50 nM carboxyfluorescein succinimidyl ester (CFSE) or CELL TRACE VIOLET (CTV) dye. Where relevant, expression of the surrogate EGFRt marker was detected by staining with an anti-EGFR antibody. Cells were seeded at a fixed number of cells per well in wells previously coated with 10, 5, 2.5, or 1.25 µg/ml of OKT3 (anti-CD3 antibody), or of anti-idiotypic antibodies recognizing SJ25C1 and FMC63 (anti-ID A-1 and anti-ID B-1, respectively) in sodium carbonate/bicarbonate buffer (pH 9.0) overnight. Cells seeded in wells with no antibody coating were included as negative controls. The cells were cultured for 4 days and assessed for viability, proliferation, and expression of CD69 and CD25.

[0568] Proliferation of anti-CD19 (SJ25C1 or FMC63) CAR-expressing T cells was assessed by dye dilution using flow cytometry. The percent of CD3⁺/CFSE^{low} cells observed following stimulation of anti-CD19 (SJ25C1) CAR-expressing T cells with anti-ID A-1 is shown in FIG. 2A. The percent of CD3⁺/EGFR⁺/CFSE^{low} cells observed following stimulation of anti-CD19 (FMC63) CAR-expressing T cells with anti-ID B-1 is shown in FIG. 2B. Incubation in the presence of the SJ25C1-derived scFv-specific anti-ID A-1 antibody was observed to lead to proliferation of anti-CD19 (SJ25C1) CAR-expressing T cells at levels

comparable to stimulation with the anti-CD3 antibody (FIG. 2A). In contrast, proliferation observed in anti-CD19 (SJ25C1) CAR-expressing T cells incubated in the presence of the other anti-Id specific for a different anti-CD19 CAR binding domain, the FMC63-derived scFv-specific anti-ID B-1) was similar to that observed in cells in the absence of any stimulatory agent. Similarly, incubation in the presence of the FMC63-derived scFv-specific anti-idiotypic antibody anti-ID B-1 was observed to result in proliferation of anti-CD19 (FMC63-derived binding domain) CAR-expressing T cells at levels comparable to stimulation with the anti-CD3 antibody. By contrast, cells incubated in the presence of another anti-Id recognizing a different anti-CD19 CAR (the SJ25C1-derived scFv-specific anti-ID A-1 antibody) was similar to that observed for cells incubated in the absence of stimulatory agents (FIG. 2B). These results demonstrated that each of the anti-ID A-1 and anti-ID B-1 was able to both stimulate and induce proliferation of T cells expressing a CAR containing the respective scFv for which the antibody was specific, and did not induce proliferation of T cells expressing a different CAR directed to the same antigen but not containing the specific binding domain to which the antibody was specific. The results are consistent with the ability of the anti-ID A-1 and anti-ID B-1 to specifically recognize their respective targets and not CARs containing other binding domains against the same antigen.

[0569] Results of another assay confirmed the ability of exemplary anti-IDs assessed in this assay to provide a CAR-spec signal to T cells in an anti-ID concentration-specific manner, consistent with the utility of anti-IDs to tune the amount of signal received via the CD19-specific CAR-expressing T cells. In this assay, mock transduced and CAR transduced T cells were labeled with CELL TRACE VIOLET (CTV). Prior to seeding, wells were coated with 0.25, 0.5, or 1 µg/ml of an exemplary anti-idiotypic antibody specific for the binding domain of the anti-CD19 CAR. The cells were cultured for 4 days and proliferation was assessed by assessing degree of dye dilution via flow cytometry. FIG. 2C shows an anti-idiotypic concentration-dependent induction of proliferation of CD8+ CAR T cells. These results demonstrate the ability to tune the amount of plate-bound exemplary anti-idiotypic antibodies provided herein, for example, to tune the amount of signal and/or provide controlled level of stimulation and/or optimize degree of stimulation, of CAR-expressing T cells in various contexts.

[0570] To further probe the stimulatory capacity of anti-ID A-1 and anti-ID B-1, T cells transduced with a target anti-CD19 CAR (SJ25C1- or FMC63-derived, respectively) were assessed using flow cytometry for expression of two markers of T cell activation, CD69 and CD25, following stimulation using plate-bound antibodies at concentrations including 1.25, 2.5, 5, and 10 $\mu\text{g/ml}$. Transduced T cells were gated for EGFR as a surrogate for CAR expression, and CD4⁺ or CD8⁺ EGFR⁺ cells were assessed for CD69 or CD25 expression.

[0571] As shown in FIG. 3 for T cells transduced with an SJ25C1-derived anti-CD19 CAR, anti-ID A-1 induced greater expression of CD25 in both CD4⁺ and CD8⁺ T cells than in cells stimulated with the anti-CD3 antibody. Anti-ID A-1 also induced the expression of CD69 in CD4⁺ and CD8⁺ T cells at levels that were similar or slightly less than when cells were stimulated with the anti-CD3 antibody. Expression of CD69 and CD25 was not observed in unstimulated cells or in cells treated with the FMC63-derived scFv-specific anti-ID B-1. As shown in FIG. 4 for T cells transduced with an FMC63-derived anti-CD19 CAR, anti-ID B-1 induced greater expression of CD25 in both CD4⁺/EGFR⁺ T cells and CD8⁺/EGFR⁺ T cells than in cells stimulated with the anti-CD3 antibody. Compared to stimulation with anti-CD3 antibody, the expression of CD69 also was induced at greater levels with the anti-ID B-1 antibody in CD4⁺/EGFR⁺ T cells and was induced at levels that were similar or slightly higher in CD8⁺/EGFR⁺ T cells. The expression of CD69 was similar or slightly higher at greater antibody concentrations in CD8⁺/EGFR⁺ T cells. Expression of CD69 and CD25 was not observed in unstimulated cells or in cells treated with the SJ25C1-specific anti-ID A-1 antibody. These results indicate that both anti-ID A-1 and anti-ID B-1 were able to specifically stimulate T cells expressing a CAR containing their target scFv.

Example 4 Analysis of transgene product-specific host immune responses

[0572] A bridge anti-therapeutic antibody (ATA) assay was developed to detect the presence or absence of antibodies, in serum of treated subjects, recognizing administered anti-CD19 CARs. Certain anti-ID antibodies described in Examples 1-3 were used as positive controls and to validate the ability of the assay to detect the presence of such antibodies.

[0573] A biotinylated human Fc-fusion protein (biotinylated ECD fusion protein) was used, containing an scFv portion of the CAR with variable regions derived from FMC63 (set forth in

SEQ ID NO:34). The biotinylated ECD fusion protein was added to streptavidin-coated wells; the plates were incubated under conditions to allow binding of the fusion protein, and washed. To the biotinylated fusion protein-coated wells, various concentrations of anti-ID B-1 or anti-ID B-2 were added to the wells, and allowed to incubate under conditions to allow specific binding of the antibody. After washing, a sulfo-tagged version of the ECD fusion protein (sulfo-ECD fusion protein) containing the FMC63-derived Fc-scFv fusion. The wells were washed and an electrochemiluminescence (ECL) signal was read on a Meso Scale Discovery (MSD) Sector Imager.

[0574] As shown in FIG. 5, the ECL signal increased as the concentration of the anti-ID B-1 and anti-ID B-2 were increased, indicating that the assay could be used to assess presence or absence and levels of antibody in serum samples, with either of the exemplary anti-ID antibodies used as a positive control.

[0575] In some embodiments, the ATA assay, using anti-ID B-1 and/or anti-ID B-2 antibody as a positive control, is used to assess the presence or absence of ATA antibodies against CAR in samples from subjects having received infusion of a dose of a cell therapy containing anti-CD19 (FMC63) CAR-expressing T cells. Such ATA may in some contexts potentially be indicative of a host humoral immune response against administered CAR. In an exemplary assay, plasma samples are obtained from subjects at various time-points, such as prior to infusion and/or on days 14 and day 28, and, in some cases, at 3, 6 and/or 12 months, after initiation of administration of the cell therapy. Samples derived from such plasma samples are used alongside control samples containing the anti-ID antibodies, in an assay such as described above.

[0576] A similar bridge assay also was generated to assess samples from subjects having received infusion of a dose of a cell therapy containing anti-CD19 (SJ25C1) CAR-expressing T cells. In this assay, the anti-ID A-1 antibody was used as a positive control. The assay was observed to have a sensitivity of less than 100 ng/mL as determined based on the positive control anti-ID antibody and >4- to 5-fold signal over plasma background.

Example 5 Conjugation of Anti-Idiotypic Antibody to Beads

[0577] Either anti-ID B-1 or Anti-ID-B1 as described in Example 2 was covalently coupled to the surface of commercially available tosyl-activated magnetic beads (ThermoFisher, Waltham MA) that are superparamagnetic, non-porous, monodisperse, tosylactivated beads. The beads covalently bind primary amino and sulfhydryl groups. Conjugation was performed using beads having a diameter of approximately 2.8 μm (designated M-280) or 4.5 μm (designated M-450).

[0578] 200 μg anti-ID antibody was added to approximately 1 mL of the tosyl-activated beads (e.g. approximately 4×10^9 tosyl-activated beads having a diameter of 2.8 μm or about 4×10^8 tosylactivated beads having a diameter of 4.5 μm) and covalent coupling was performed by overnight incubation at 37°C in phosphate buffered solution (PBS) containing 0.1% human serum albumin (HSA). Beads were washed and resuspended in 1 mL PBS with 0.1% HSA. After conjugation, the bead concentration was determined using a Cellometer.

[0579] To assess stability of the anti-ID conjugated beads, the beads were pelleted, the supernatant was removed and loaded on a 4-12% Bis-Tris SDS-PAGE gel and the gel was stained with Coomassie blue. As a control for total protein conjugated on the beads, the pelleted beads were boiled in 4X (lithium dodecyl sulfate) LDS sample buffer at about 70° C for 20 minutes and approximately 12.5 μL or 25 μL boiled supernatant also was run on SDS-PAGE gel and assessed by Coomassie blue. Approximately 2.5 μg or 5.0 μg anti-ID antibody that had not been conjugated to the beads (positive control) or 5 μL 0.1% HSA (negative control) also were assessed by SDS-PAGE and Coomassie staining. No anti-ID antibody was detected in supernatant from conjugated samples that had not been boiled indicating that the conjugation was stable, whereas anti-ID antibody was detected in supernatant from conjugated samples that were boiled.

Example 6 Assessment of stimulation of T cells cultured with anti-idiotypic antibody-conjugated beads

[0580] The FMC63-derived scFv-specific anti-ID B-1- conjugated beads were incubated with T cells. CD3-purified T cells were isolated by immunoaffinity-based enrichment from leukapheresis samples from healthy donors. Isolated cells were transduced with a viral vector

encoding an anti-CD19 CAR having an scFv derived from FMC63. The viral vector construct further encoded a truncated EGFR (EGFRt), which served as a surrogate marker for CAR expression; the EGFRt-coding region was separated from the CAR sequence by a T2A skip sequence. After transductions, cells were expanded in culture and frozen by cryopreservation.

[0581] For T cells stimulation studies, thawed CD4+ or CD8+ CAR-expressing cells were separately seeded at approximately 50,000 total cells per well. In some cases, the culture media was additionally supplemented with cytokines as follows: for CD4+ cells, approximately 1200 IU/mL recombinant IL-7, 20 IU/mL recombinant IL-15 and 100 IU/mL recombinant IL-2; for CD8+ cells, approximately 200 IU/mL recombinant IL-2 and 20 IU/mL recombinant IL-15. Anti-ID B-1 conjugated beads were added to cells at a 1:1 or 1:5 cell:bead ratio and incubated up to 14 days with a 50% media exchange every 2-3 days. As a positive control, cells were cultured with anti-CD3/anti-CD28 magnetic beads at a 3:1 cell:bead ratio in the presence or absence of the indicated cytokines.

[0582] At various times of culture, CD4+ or CD8+ transduced cells (as detected by anti-EGFR for expression of the surrogate marker) were assessed for expansion, PD-1 expression and viability.

[0583] As shown in FIG.6 and 7, expansion of CD4+ and CD8+ T cells, respectively, was observed when cells were cultured with anti-ID conjugated beads at either the 1:1 or 1:5 cell:bead ratio, particularly in the presence of cytokines. The extent of expansion was greater than when cells were cultured with the control anti-CD3/anti-CD28 magnetic beads.

[0584] Surface expression of PD-1 on the CD4+ cell subset was assessed by flow cytometry at days 3, 7, 10 and 14 of culture. As shown in FIG. 8, PD-1 expression was high on cells cultured with anti-CD3/anti-CD28 magnetic beads, however, the levels of PD-1 was substantially lower or undetectable in cells that were cultured with anti-ID conjugated beads.

[0585] As shown in FIG. 9, the percent viability of CD4+ and CD8+ T cells cultured with either the 1:1 or 1:5 ratio of anti-ID conjugated beads or the control anti-CD3/anti-CD28 conjugated beads remained consistently high during the period of culture when the cells were additionally cultured in the presence of cytokines. Under all conditions, however, viability of cells decreased in the absence of added cytokines, with the greatest loss in cell viability occurring at later days of cell culture.

Example 7 Assessment of cytokine production of T cells cultured with anti-idiotypic antibody-conjugated beads

[0586] T cells engineered with an anti-CD19 CAR having an scFv derived from FMC63 were generated substantially as described in Example 6. Thawed CD8⁺ T cells were cultured with anti-ID B-1 conjugated beads for 4 hours in the presence of Golgi inhibitor. Intracellular cytokine levels of TNF α , IFN γ , and IL-2 was determined by flow cytometry in either the CAR⁺ T cell subset (as determined by positive surface staining for the surrogate EGFRt transduction marker with anti-EGFR) or the CAR⁻ T cell subset (as determined by negative surface staining for EGFRt with anti-EGFR). As a comparison, CAR⁺ T cells (EGFR⁺) also were cultured with CD19-expressing target cells (K562 cells transduced to express CD19, K562-CD19) at an effector:T cell ratio of 1:2.

[0587] As shown in FIG. 10A, intracellular cytokine levels of TNF α , IFN γ and IL2 cytokines were induced in CAR⁺ T cells (EGFRt⁺), but not in CAR⁻ T cells (EGFR⁻), when the cells were cultured in the presence of the anti-ID B-1 conjugated beads. In this study, the extent of stimulation observed in the presence of anti-ID conjugated beads was similar to stimulation of CAR⁺ T cells using antigen-expressing K562-CD19 cells, which is an alternative CAR-specific stimulation reagent (FIG. 10B). These results demonstrated that anti-ID conjugated to beads are agonistic and specifically stimulate T cells expressing a CAR having an antigen-binding domain recognized by the anti-ID antibody. Further, the bead reagent provides for a better CAR-specific stimulation reagent compared to cell lines, which require cell culture and are prone to lot to lot variability.

Example 8 Assessment of Expansion after Serial Restimulation

[0588] The ability of cells to expand *ex vivo* following repeated stimulations may be a potential surrogate for capacity of CAR⁺ T cells to persist (e.g. following initial activation) and/or is indicative of function in vivo (Zhao et al. (2015) Cancer Cell, 28:415-28). CAR⁺ T cells were generated as described above and thawed CD4⁺ or CD8⁺ CAR-expressing T cells were plated separately at 50,000 CAR⁺ cells/well. Anti-ID B-1 conjugated beads were added to

cells at a 1:1 or 1:5 cell:bead ratio in the presence or absence of cytokines as described in Example 6. As a control, anti-CD3/anti-CD28 magnetic beads were added to cells at a 3:1 cell:bead ratio in the presence or absence of the cytokines. Cells were harvested every 3-4 days and counted, and restimulated with new target cells using the same culture conditions after resetting cell number to initial seeding density for each round. A total of 4 rounds of stimulation during a 14 day culture period were carried out. For each round of stimulation, the number of doublings was determined.

[0589] As shown in FIG. 11, continued cell expansion of CAR-expressing (EGFR+) CD4+ cells was observed after restimulation with anti-ID conjugated beads, although the degree of expansion was greater when the cells were cultured in the presence of cytokines. Also, the extent of expansion was greater than when cells were cultured with anti-CD3/anti-CD28 beads. For CD8+ T cells, similar expansion kinetics was observed when cells were cultured in the presence of either anti-ID conjugated beads or anti-CD3/anti-CD28 beads, although expansion was somewhat greater when cells were cultured with anti-ID conjugated beads, particularly in the absence of added recombinant cytokines.

Example 9 Further Analysis of CAR-Specific Cell Expansion Using Anti-idiotypic antibody-Conjugated Beads

[0590] Similar studies as those described in Examples 7 and 8 were performed, except using CAR-expressing T cells generated from two different patient donors. CD3-purified T cells were isolated from peripheral blood mononuclear cells (PBMCs) of two donor patients, transduced with a viral vector encoding an anti-CD19 CAR having an scFv derived from FMC63, expanded in culture, frozen, and thawed.

[0591] Thawed CD4+, CD8+ or co-cultures of CD4/CD8 (1:1 ratio) were seeded in wells of a 6-well plate at approximately 5×10^6 total cells/well in culture media that was additionally supplemented with cytokines as follows: for CD4+ cells or CD4+/CD8+ co-cultures media was supplemented with approximately 1200 IU/mL recombinant IL-7, 20 IU/mL recombinant IL-15 and 100 IU/mL recombinant IL-2; for CD8+ cells media was supplemented with 200 IU/mL recombinant IL-2 and 20 IU/mL recombinant IL-15. Anti-ID B-1 conjugated beads were added

to cells at a 1:1 cell:bead ratio and incubated up to 9 days with a 50% media exchange every 2-3 days.

[0592] At various times of culture, the number of CD4+ or CD8+ transduced cells (as detected by anti-EGFR for expression of the surrogate marker) present in the culture for each condition was assessed and fold expansion or frequency of the CAR-expressing cells as a percent of total cells was determined. Expression of PD-1 and CD25 and cell viability also was determined.

[0593] As shown in FIG. 12A, over 60-fold expansion of CAR-expressing (EGFRt+) CD4+ T cells was observed when CD4+ cells were cultured alone with anti-ID conjugated beads. For CD8+ T cells, substantially higher expansion of CAR-expressing (EGFRt+) CD8+ T cells occurred in the presence of anti-ID conjugated beads when the CD8+ T cells were co-cultured with CD4+ cells. As shown in FIG. 12B, the frequency of CAR-expressing (EGFRt+) CD4+ or CD8+ increased during the 9 days of culture in the presence of the anti-ID conjugated beads with >90% of transduced cells (EGFRt+) present in the culture at day 9. Viability of CD4+ and CD8+ cells also remained close to 100% during the culture, with somewhat greater viability of CD8+ T cells observed when co-cultured with CD4+ T cells (FIG. 12C). Similar results were observed for both donors.

[0594] Surface expression of PD-1 and CD25 on transduced (EGFRt+) CD4+ or CD8+ cells was assessed by flow cytometry at days 5, 7 and 9 of culture with the anti-ID conjugated beads. As shown in FIG. 13A, PD-1 expression on both CD4+ and CD8+ T cells decreased substantially over time of cultured with anti-ID conjugated beads. As shown in FIG. 13B, CD25 expression also was decreased after 9 days of culture in the presence of anti-ID conjugated beads. Similar results were observed for both donors.

Example 10 Comparison of Cytokine Levels and Phenotype After Culture with Anti-Idiotypic Antibody Conjugated Beads

[0595] CD3-purified T cells were isolated from peripheral blood mononuclear cells (PBMCs) of two donor patients, transduced with a viral vector encoding an anti-CD19 CAR having an scFv derived from FMC63 and expanded in culture either with anti-CD3 and CD28

antibody coated beads. After expansion, the expanded T cells were frozen by cryopreservation. For the studies, frozen T cells were thawed and CD4+ and CD8+ T cells assessed for intracellular cytokine levels or surface phenotype (d=0) or thawed CD4+, CD8+, or CD4+/CD8+ co-culture T cells were cultured for an additional 9 days in the presence of anti-ID B-1 conjugated beads prior to assessing intracellular cytokine levels and surface marker phenotype following stimulation by PMA/ionomycin or CD19 transduced K562 cells (d=9).

[0596] For assessment of intracellular cytokine levels, Golgi inhibitor was added for 4 hours and then TNF α , IFN γ , and IL-2 was assessed by flow cytometry. For all conditions, the extent of intracellular cytokine expression was substantially greater when cells were stimulated with PMA/ionomycin as compared to CAR-specific stimulation with CD19 transduced K562 cells. As shown in FIG. 14A, the levels of TNF α and IL-2 cytokines were similar in CD4+ or CD8+ cells immediately after thaw compared to corresponding CD4+ or CD8+ cells, or co-cultures of CD4/CD8 T cells, further cultured in the presence of anti-ID conjugated beads for 9 days. Increased levels of IFN γ was observed in thawed CD4+, CD8+ or CD4/CD8 co-culture T cells that were further cultured in the presence of anti-ID conjugated beads for 9 days compared to the level of IFN γ in CD4+ or CD8+ T cells immediately after thaw. These results demonstrated that T cell function was maintained following 9 day expansion with anti-ID conjugated beads. Similar results were obtained in cells from the two donors.

[0597] Surface expression of the activation marker CD25, surface expression of inhibitory receptors PD-1 and LAG-3 and nuclear expression of the proliferation marker Ki-67 were also assessed in CD4+ or CD8+ cells immediately after thaw (d=0) or in CD4+ or CD8+ T cells expanded alone or as a CD4/CD8 co-culture for an additional 9 days in the presence of the anti-ID conjugated beads (d=9). As shown in FIG. 14B, reduced expression of CD25, but not Ki-67, was observed in cells that were further cultured in the presence of anti-ID conjugated beads for 9 days compared to T cells immediately after thaw. The reduced expression of CD25 was substantially greater in CD8+ cells than in CD4+ cells. In addition, decreased expression of PD-1 and LAG-3 also was observed in both CD4+ and CD8+ cells cultured alone or as a CD4/CD8 co-culture after incubation for 9 days with anti-ID conjugated beads compared to expression in cells immediately after thaw. This result demonstrated that after incubation with the anti-ID conjugated beads, the previously frozen transduced cells retained functional ability as evident by

the high percentage of cells that were positive for the marker Ki-67, indicative of cell proliferation, but also exhibited a different activation state characterized by the low surface expression for the CD25 activation marker and the inhibitory receptor markers PD-1 and LAG-3.

Example 11 Detection of CAR-Expressing Cells With Anti-Idiotypic Antibodies.

[0598] T cell compositions were generated containing CAR-T cells expressing an anti-CD19 CAR containing an anti-CD19 scFv with variable regions derived from the FMC63 antibody, an immunoglobulin spacer, a transmembrane domain derived from CD28, a costimulatory region derived from 4-1BB, and a CD3-zeta intracellular signaling domain. The viral construct encoding the CAR further encoded an EGFRt surrogate marker that was separated from CAR sequence by a T2A skip sequence.

[0599] The anti-CD19 CAR expressing cells were spiked in to a sample of cells containing healthy human peripheral blood mononuclear cells (PBMCs). The resulting cell compositions were incubated with different concentrations of the anti-CD19 FMC63 scFv-specific anti-ID B1 or anti-ID B2 antibodies. As a control, samples of each condition were also incubated with a concentration of anti-EGFR antibody, capable of detecting the EGFRt surrogate marker on the transduced cells. Control samples included those only containing the PBMCs without the addition of added CAR-expressing T cells. Geometric mean fluorescence intensity (MFI) was quantified in positively labeled cells to assess antibody staining.

[0600] As shown in FIG. 15A, both anti-idiotypic antibodies detected the T cells with the anti-CD19 CAR having an scFv with variable regions derived from FMC63, in a concentration-dependent manner. As shown in FIG. 15B, the anti-ID B1 and anti-ID B2 antibodies detected positive cells in the compositions that contained CAR expressing cells and not in compositions that contained only PBMCs. Staining for EGFRt indicated that the ratio of cells expressing the transgene to PBMCs was consistent across all conditions. The results confirmed the ability of the anti-ID B1 and anti-ID B2 antibodies to specifically detect anti-CD19 FMC63 scFv CAR-expressing cells, even among samples containing human PBMCs that did not express a CAR. The results are consistent with an interpretation that the anti-idiotypic antibodies may be used to detect CAR expressing cells in samples from subjects having been administered the CAR-

expressing cells recognized by the antibodies, such as peripheral blood samples collected from human subjects, for example to measure expansion, trafficking, and/or persistence of such cells over time in various tissues and/or fluids of the subject.

[0601] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

SEQUENCES

#	SEQUENCE	ANNOTATION
1	QVQLQQPGSELVRPGGSVKLSCKASDYTFSTSYWMHWVRQRPGQ GLEWIGNIYPGSGGTNYDEKFKRKATLTVDTSSSTAYMQLRSLTS EDSAVYYCTREVTTVAYYYSM DYWGQGTSTVTVSS	anti-ID VH
2	AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGS LSSGVHTFPAVLQSDLYTLSSSVTPSSTWPSETVTCNVAHPASST KVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITITLTPKVT VVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIP PPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPI MDTDGSYFVYSKLVNQQSNWEAGNTFTCSVLHEGLHNHHTKSL SHSPGK	anti-ID CH
3	QVQLQQPGSELVRPGGSVKLSCKASDYTFSTSYWMHWVRQRPGQ GLEWIGNIYPGSGGTNYDEKFKRKATLTVDTSSSTAYMQLRSLTS EDSAVYYCTREVTTVAYYYSM DYWGQGTSTVTVSSAKTTPPSVYP LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTPSSTWPSETVTCNVAHPASSTKVDKKIVPRD CGCKPCICTVPEVSSVFIFPPKPKDVLITITLTPKVTCTVVVDISKDDP EVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLN GKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKD KVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFV YSKLVNQQSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK	anti-ID heavy chain
4	MGWSSIILFLVATASGVHS	anti-ID HC signal sequence
5	DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVK LLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGK TVPFTFGSGTKLEIK	anti-ID VL
6	RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGS ERQNGVLNSWTDQDSKDSTYSMSSTLTTLTKDEYERHNNYTCEAT HKTSTSPIVKSFNRECE	anti-ID CL
7	DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVK LLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGK TVPFTFGSGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNF YPKDINVKWKIDGSRQNGVLNSWTDQDSKDSTYSMSSTLTTLTK DEYERHNNYTCEATHKTSTSPIVKSFNRECE	anti-ID light chain
8	MMSSAQFLGLLLLCFQGTRC	anti-ID LC signal sequence
9	SYWMH	anti-ID HC-CDR1
10	NIYPGSGGTNYDEKFKR	anti-ID HC-CDR2
11	EVTTVAYYYSM DY	anti-ID HC-CDR3
12	RASQDISNYLN	anti-ID LC-CDR1
13	YTSRLHS	anti-ID LC-CDR2

14	QQGKTVPF	anti-ID LC-CDR3
15	CAGGTCCAACCTGCAACAACCTGGGTCTGAGCTGGTGAGGCCTG GAGGTTTCAGTGAAGCTGTCCTGCAAGGCTTCTGACTACACTTTC ACCAGCTACTGGATGCACTGGGTGAGGCAGAGGCCTGGACAA GGCCTTGAGTGGATTGGAAATATTTATCCTGGTAGTGGTGGTA CTAACTACGATGAGAAGTTCAAGAGGAAGGCCCACTGACTGT AGACACATCCTCCAGCACAGCCTACATGCAGCTCCGCAGCCTG ACATCTGAGGACTCTGCGGTCTATTACTGTACAAGAGAGGTTA CTACAGTAGCTTATTACTATTCTATGGACTACTGGGGTCAAGG AACCTCAGTCACCGTCTCCTCA	anti-ID VH
16	GCCAAAACGACACCCCATCTGTCTATCCACTGGCCCCTGGAT CTGCTGCCAACTAACTCCATGGTGACCCTGGGATGCCTGGT CAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTCT GGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCTGCA GTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCA GCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCACCC GGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGA TTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCAT CTGTCTTCATCTTCCCCCAAAGCCCAAGGATGTGCTCACCATT ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCA AGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGT GGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTT CAACAGCACTTTCCGCTCAGTCAGTGAACCTTCCCATCATGCACC AGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACA GTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAAC CAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGC ATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGC AGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACTCAGC CCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCT CAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTACAC CTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATCTGAG AAGAGCCTCTCCCACTCTCCTGGTAAATGA	anti-ID CH
17	CAGGTCCAACCTGCAACAACCTGGGTCTGAGCTGGTGAGGCCTG GAGGTTTCAGTGAAGCTGTCCTGCAAGGCTTCTGACTACACTTTC ACCAGCTACTGGATGCACTGGGTGAGGCAGAGGCCTGGACAA GGCCTTGAGTGGATTGGAAATATTTATCCTGGTAGTGGTGGTA CTAACTACGATGAGAAGTTCAAGAGGAAGGCCCACTGACTGT AGACACATCCTCCAGCACAGCCTACATGCAGCTCCGCAGCCTG ACATCTGAGGACTCTGCGGTCTATTACTGTACAAGAGAGGTTA CTACAGTAGCTTATTACTATTCTATGGACTACTGGGGTCAAGG AACCTCAGTCACCGTCTCCTCAGCCAAAACGACACCCCATCT GTCTATCCACTGGCCCCTGGATCTGCTGCCAACTAACTCCAT GGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCA GTGACAGTGACCTGGAACCTCTGGATCCCTGTCCAGCGGTGTGC ACACCTTCCCAGCTGTCTGCACTGTGACCTCTACACTCTGAGC AGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCG TCACCTGCAACGTTGCCACCCGGCCAGCAGCACCAGGTGGA CAAGAAAATTGTGCCAGGGATTGTGGTTGTAAGCCTTGCATA	anti-ID heavy chain

	TGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCAAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACG TGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGT TCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGAC GCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTC AGTGAACCTCCCATCATGCACCAGGACTGGCTCAATGGCAAGG AGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCTGCCCCCAT CGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCC ACAGGTGTACACCATTCACCTCCCAAGGAGCAGATGGCCAAG GATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTG AAGACATTACTGTGGAGTGGCAGTGGAAATGGGCAGCCAGCGG AGAATAACAAGAACTCAGCCCATCATGGACACAGATGGCTC TTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAACTGG GAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCC TGCACAACCACATACTGAGAAGAGCCTCTCCCACTCTCCTGG TAAATGA	
18	ATGGGATGGAGCTCTATCATCCTCTTCTTGGTAGCAACAGCCTC AGGTGTCCACTCC	anti-ID HC signal sequence
19	GATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCT GGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACAT TAGCAATTATTTAAACTGGTATCAGCAGAAACCAGATGGAACT GTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAG TCCCATCAAGGTTCAAGTGGCAGTGGGTCTGGAACAGATTATTC TCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTAC TTTTGTCAGCAGGGTAAAACGGTTCCATTACGTTTCGGCTCGG GGACAAAGTTGGAAATAAAA	anti-ID VL
20	CGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAG TGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGA ACAACCTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGA TGGCAGTGAACGACAAAAATGGCGTCCTGAACAGTTGGACTGAT CAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCCTC ACGTTGACCAAGGACGAGTATGAACGACATAACAACCTATACCT GTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAG CTTCAACAGGAATGAGTGTTAG	anti-ID CL
21	GATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCT GGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACAT TAGCAATTATTTAAACTGGTATCAGCAGAAACCAGATGGAACT GTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAG TCCCATCAAGGTTCAAGTGGCAGTGGGTCTGGAACAGATTATTC TCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTAC TTTTGTCAGCAGGGTAAAACGGTTCCATTACGTTTCGGCTCGG GGACAAAGTTGGAAATAAAAACGGGCTGATGCTGCACCAACTGT ATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGT GCCTCAGTCGTGTGCTTCTTGAACAACCTTCTACCCCAAAGACAT CAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAAATGG CGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACC TACAGCATGAGCAGCACCCCTCACGTTGACCAAGGACGAGTATG AACGACATAACAACCTATACCTGTGAGGCCACTCACAAGACATC AACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG	anti-ID light chain

22	ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTT CAAGGTACCAGATGT	anti-ID LC signal sequence
23	EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQG LEWIGQIYPGDGDTNYNGKFKGQATLTADKSSSTAYMQLSGLTSE DSAVYFCARKTISSVVDYFDYWGQGTTVTVSS	SJ25C1 VH
24	DIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSP KPLIYSATYRNSGVPDRFTGSGSGTDFTLTITNVQSKDLADYFCQQ YNRYPYTSGGGTKLEIKR	SJ25C1 VL
25	GGGSGGGSGGGGS	Linker
26	GGGS	4GS linker
27	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP	An extracellular portion of CD28
28	EVKLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQG LEWIGQIYPGDGDTNYNGKFKGQATLTADKSSSTAYMQLSGLTSE DSAVYFCARKTISSVVDYFDYWGQGTTVTVSSGGGSGGGSGG GGSDIELTQSPKFMSTSVGDRVSVTCKASQNVGTNVAWYQQK GQSPKPLIYSATYRNSGVPDRFTGSGSGTDFTLTITNVQSKDLADY FCQQYNRYPYTSGGGTKLEIKR	SJ25C1 scFv
29	GGGS	3GS linker
30	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGL EWLGVIWGSETTYYNALKSRLTIKDNSKSQVFLKMNSLQTDDT AIYYCAKHYYGGSYAMDYWGQGTSTVTVSS	FMC63 VH
31	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVK LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN TLPYTFGGGKLEIT	FMC63 VL
32	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	IgG1 Fc lacking hinge
33	GSTSGSGKPGSGEGSTKG	Linker
34	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVK LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN TLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVA PSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTY YNALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYGG SYAMDYWGQGTSTVTVSS	FMC63 scFv
35	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVK LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN TLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVA PSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTY YNALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYGG SYAMDYWGQGTSTVTVSSPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGK	FMC63 reagent
36	EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMKWVKQCHGK	anti-ID B-1 VH

	SLEWIGDINPNNGGTDYNQNFKGKATLTVDKSSSTAYMQLNSLTS EDSAVYYCAREGNNYGSRDAMDYWGQGTSTVTVSS	
37	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGS LSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASST KVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLS PIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTL RVVVSALPIQH QDWMMSGKEFKCKVNNKDLPAPIERTISKPKGSVRA PQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTEL NYKNTEPVLDS DGSYFMYSKLRVEKKNWVERNSYSCSVVHEGL HNHHTTKSFSRTPGK	anti-ID B-1 CH
38	EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMKWVKQCHGK SLEWIGDINPNNGGTDYNQNFKGKATLTVDKSSSTAYMQLNSLTS EDSAVYYCAREGNNYGSRDAMDYWGQGTSTVTVSSAKTTAPSVY PLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPR GPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVD VSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMMSGKEFKCKVNNKDLPAPIERTISKPKGSVRA PQVYVLPPE EEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVL DSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFS RTPGK	anti-ID B-1 heavy chain
39	MGWSWIFLFLLSGTAGVLS	anti-ID B-1 HC signal sequence
40	QIVLTQSPALMSASPGEKVTMTCSASSGVIYMYWYQQKPRSSPKP WIYLTSLNLSGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQW SSNPLTFGAGTKLELK	anti-ID B-1 VL
41	RADAAPTVSIFPPSSEQLTSGGASVVCFLN NFYPKDINVKWKIDGS ERQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEAT HKTSTSPIVKSFNRNEC	anti-ID B-1 CL
42	QIVLTQSPALMSASPGEKVTMTCSASSGVIYMYWYQQKPRSSPKP WIYLTSLNLSGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQW SSNPLTFGAGTKLELK RADAAPTVSIFPPSSEQLTSGGASVVCFLN NFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDYSTYSMSSTLT TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC	anti-ID B-1 light chain
43	MDFQVQIFSFLMSASVIMSRG	anti-ID B-1 LC signal sequence
44	DYYMK	anti-ID B-1 HC- CDR1
45	DINPNNGGTDYNQNFKG	anti-ID B-1 HC- CDR2
46	EGNNYGSRDAMDY	anti-ID B-1 HC- CDR3
47	SASSGVIYMY	anti-ID B-1 LC- CDR1
48	LTSNLAS	anti-ID B-1 LC- CDR2
49	QQWSSNPLT	anti-ID B-1 LC- CDR3
50	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTGAAGCCTG	anti-ID B-1 VH

	GGGCTTCAGTGAAGATGTCCTGTAAGGCTTCTGGATACACATT CACTGACTACTACATGAAGTGGGTGAAGCAGTGTGCATGGAAAG AGCCTTGAGTGGATTGGAGATATTAATCCTAACAATGGTGGTA CTGACTACAACCAGAACTTTAAGGGCAAGGCCACATTGACTGT AGACAAATCCTCCAGCACAGCCTACATGCAGCTCAACAGCCTG ACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAGAGGGGA ATAACTACGGTAGTAGAGATGCTATGGACTACTGGGGTCAAGG AACGTCAGTCACCGTCTCCTCA	
51	GCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCCTGTGT GTGGAGATACAACCTGGCTCCTCGGTGACTCTAGGATGCCTGGT CAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCT GGATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCA GTCTGACCTCTACACCCTCAGCAGCTCAGTGACTGTAACCTCG AGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCCACC CGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAG GGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCACCT TAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGA TCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGT GTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCA GCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACA AACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGT GCCCTCCCCATCCAGCACAGGACTGGATGAGTGGCAAGGAGT TCAAATGCAAGGTCAACAACAAGACCTCCCAGCGCCCATCGA GAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACA GGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAA CAGGTCACTCTGACCTGCATGGTCAACAGACTTCATGCCTGAAG ACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAA ACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTA CTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAAGTGGGT GGAAAGAAATAGCTACTCCTGTTCAAGTGGTCCACGAGGGTCTG CACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTA AA	anti-ID B-1 CH
52	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTGAAGCCTG GGGCTTCAGTGAAGATGTCCTGTAAGGCTTCTGGATACACATT CACTGACTACTACATGAAGTGGGTGAAGCAGTGTGCATGGAAAG AGCCTTGAGTGGATTGGAGATATTAATCCTAACAATGGTGGTA CTGACTACAACCAGAACTTTAAGGGCAAGGCCACATTGACTGT AGACAAATCCTCCAGCACAGCCTACATGCAGCTCAACAGCCTG ACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAGAGGGGA ATAACTACGGTAGTAGAGATGCTATGGACTACTGGGGTCAAGG AACGTCAGTCACCGTCTCCTCAGCCAAAACAACAGCCCCATCG GTCTATCCACTGGCCCCCTGTGTGTGGAGATACAACCTGGCTCCTC GGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCA GTGACCTTGACCTGGAACCTCTGGATCCCTGTCCAGTGGTGTGC ACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTCAGC AGCTCAGTGACTGTAACCTCGAGCACCTGGCCCAGCCAGTCCA TCACCTGCAATGTGGCCCACCCGGCAAGCAGCACCAAGGTGGA CAAGAAAATTGAGCCCAGAGGGCCACAATCAAGCCCTGTCCT CCATGCAAATGCCCAGCACCTAACCTCTTGGGTGGACCATCCG	anti-ID B-1 heavy chain

	TCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCC CTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGG ATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGA AGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAA CAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAG GACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAAC AAAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCA AAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACC AGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCAT GGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACC AACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCA GTCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAG AGTGGAAGAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTG TTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAG AGCTTCTCCCGGACTCCGGGTAAA	
53	ATGGGATGGAGCTGGATCTTTCTCTTCTCTTGTGTCAGGAAGTGC AGGTGTCCTCTCT	anti-ID B-1 HC signal sequence
54	CAAATTGTTCTCACCCAGTCTCCAGCACTCATGTCTGCATCTCC AGGGGAGAAGGTCACCATGACCTGCAGTGCCAGCTCAGGTGTA ATTTACATGTACTGGTACCAACAGAAGCCAAGATCCTCCCCCA AACCCTGGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCT GCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCAC AATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGC CAGCAGTGGAGTAGTAACCCGCTCACGTTCCGGTGCTGGCACCA AGCTGGAGCTGAAA	anti-ID B-1 VL
55	CGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAG TGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGA ACAACCTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGA TGGCAGTGAACGACAAAAATGGCGTCCTGAACAGTTGGACTGAT CAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCCTC ACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCT GTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAG CTTCAACAGGAATGAGTGT	anti-ID B-1 CL
56	CAAATTGTTCTCACCCAGTCTCCAGCACTCATGTCTGCATCTCC AGGGGAGAAGGTCACCATGACCTGCAGTGCCAGCTCAGGTGTA ATTTACATGTACTGGTACCAACAGAAGCCAAGATCCTCCCCCA AACCCTGGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCT GCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCAC AATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGC CAGCAGTGGAGTAGTAACCCGCTCACGTTCCGGTGCTGGCACCA AGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTATCCAT CTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCA GTCGTGTGCTTCTTGAACAACCTTCTACCCCAAAGACATCAATGT CAAGTGGAAGATTGATGGCAGTGAACGACAAAAATGGCGTCCT GAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAG CATGAGCAGCACCCCTCACGTTGACCAAGGACGAGTATGAACGA CATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTT CACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT	anti-ID B-1 light chain
57	ATGGATTTTCAAGTGCAGATTTTCAGCTTCTGCTAATGAGTGC	anti-ID B-1 LC

	CTCAGTCATAATGTCCAGGGGA	signal sequence
58	QVQLQQPGAELVRPGASVKLSCKTSGYSFTRYWMNWVKQRPGQ GLEWIGMIHPSDSETRLNQKFKDKATLTVDNSSSTAYMQLSSPTS EDSAVYYCASIYYEEAWGQGTLVTVSA	anti-ID B-2 VH
59	AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGS LSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNV AHPASST KVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVT VVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNSA AAFPAPIEKTISKTKGRPKAPQVYTIP PPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPI MDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLHEGLHNHHTTEKSL SHSPGK	anti-ID B-2 CH
60	QVQLQQPGAELVRPGASVKLSCKTSGYSFTRYWMNWVKQRPGQ GLEWIGMIHPSDSETRLNQKFKDKATLTVDNSSSTAYMQLSSPTS EDSAVYYCASIYYEEAWGQGTLVTVSAAKTTPPSVYPLAPGSAA QTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDL YTLSSSVTVPSSTWPSETVTCNV AHPASSTKVDKKIVPRDCGCKPC ICTVPEVSSVFIFPPKPKDVLITLTPKVT CVVVDISKDDPEVQFSW FVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCR VNSA AAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCM ITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQ KSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK	anti-ID B-2 heavy chain
61	MGWSSIILFLVATATGVHS	anti-ID B-2 HC signal sequence
62	DIQMTQSPASLSASVGETVTITCRASGNIHNYLA WYQQKQKGKSPQ LLVYN AKTLADSVPSRFSGSGSGTQYSLKINS LQPEDFGSY YCQHF WSTPYTFGGGTKLEIK	anti-ID B-2 VL
63	DIQMTQSPASLSASVGETVTITCRASGNIHNYLA WYQQKQKGKSPQ LLVYN AKTLADSVPSRFSGSGSGTQYSLKINS LQPEDFGSY YCQHF WSTPYTFGGGTKLEIKRADAAPT VSIFFPSSEQLTSGGASVVCFLN NFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTL TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC	anti-ID B-2 light chain
64	MSVLTQVLALLLLWLTGARC	anti-ID B-2 LC signal sequence
65	RYWMN	anti-ID B-2 HC- CDR1
66	MIHPSDSETRLNQKFKD	anti-ID B-2 HC- CDR2
67	IYYEEA	anti-ID B-2 HC- CDR3
68	RASGNIHNYLA	anti-ID B-2 LC- CDR1
69	NAKTLAD	anti-ID B-2 LC- CDR2
70	QHFWSPTYT	anti-ID B-2 LC- CDR3
71	CAGGTCCA ACTGCAGCAGCCTGGGGCTGAGCTGGTGAGGCCTG GAGCTTCAGTGAAGCTGTCCTGCAAGACTTCTGGCTACTCCTC ACCAGGTACTGGATGAAGTGGGTGAAGCAGAGGCCTGGACAA	anti-ID B-2 VH

	GGCCTTGAGTGGATTGGCATGATTCATCCTTCCGATAGTGAAA CTAGGTTAAATCAGAAGTTCAAGGACAAGGCCACATTGACTGT AGACAATTCTCCAGCACAGCCTACATGCAACTCAGCAGCCCCG ACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGCATCTACTA TGAAGAGGCCTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA	
72	GCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGAT CTGCTGCCCAAATACTCCATGGTGACCCTGGGATGCCTGGT CAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTCT GGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCA GTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCA GCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCACCC GGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGA TTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCAT CTGTCTTCATCTTCCCCCAAGCCCAAGGATGTGCTCACCATT ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCA AGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGT GGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTT CAACAGCACTTTCCGCTCAGTCAGTGAACCTTCCCATCATGCACC AGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACA GTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAAC CAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCACCT CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGC ATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGC AGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACTCAGC CCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCT CAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTCAC CTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAG AAGAGCCTCTCCCACTCTCCTGGTAAA	anti-ID B-2 CH
73	CAGGTCCAACCTGCAGCAGCCTGGGGCTGAGCTGGTGAGGCCTG GAGCTTCAGTGAAGCTGTCCTGCAAGACTTCTGGCTACTCCTTC ACCAGGTACTGGATGAACTGGGTGAAGCAGAGGCCTGGACAA GGCCTTGAGTGGATTGGCATGATTCATCCTTCCGATAGTGAAA CTAGGTTAAATCAGAAGTTCAAGGACAAGGCCACATTGACTGT AGACAATTCTCCAGCACAGCCTACATGCAACTCAGCAGCCCCG ACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGCATCTACTA TGAAGAGGCCTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA GCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGAT CTGCTGCCCAAATACTCCATGGTGACCCTGGGATGCCTGGT CAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTCT GGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCA GTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCA GCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCACCC GGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGA TTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCAT CTGTCTTCATCTTCCCCCAAGCCCAAGGATGTGCTCACCATT ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCA AGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGT GGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTT CAACAGCACTTTCCGCTCAGTCAGTGAACCTTCCCATCATGCACC	anti-ID B-2 heavy chain

	AGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACA GTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAAC CAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCACCT CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGC ATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGC AGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACTCAGC CCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCT CAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTCAC CTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAG AAGAGCCTCTCCCACTCTCCTGGTAAA	
74	ATGGGATGGAGCTCTATCATCCTCTTCTTGGTAGCAACAGCTAC AGGTGTCCACTCC	anti-ID B-2 HC signal sequence
75	GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGT GGGAGAACTGTCACCATCACATGTGCGAGCAAGTGGGAATATT CACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTC CTCAGCTCCTGGTCTATAATGCAAAAACCTTAGCAGATAGTGT GCCATCAAGGTTCAAGTGGCAGTGGATCAGGAACACAATATTCT CTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATT ACTGTCAACATTTTGGAGTACTCCGTACACGTTCCGGAGGGGG GACCAAGCTGGAAATAAAA	anti-ID B-2 VL
76	GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGT GGGAGAACTGTCACCATCACATGTGCGAGCAAGTGGGAATATT CACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTC CTCAGCTCCTGGTCTATAATGCAAAAACCTTAGCAGATAGTGT GCCATCAAGGTTCAAGTGGCAGTGGATCAGGAACACAATATTCT CTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATT ACTGTCAACATTTTGGAGTACTCCGTACACGTTCCGGAGGGGG GACCAAGCTGGAAATAAAAACGGGCTGATGCTGCACCAACTGTA TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTG CCTCAGTCGTGTGCTTCTTGAACAACCTTACCCCCAAAGACATC AATGTCAAGTGGAAAGATTGATGGCAGTGAACGACAAAATGGC GTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCT ACAGCATGAGCAGCACCTCACGTTGACCAAGGACGAGTATGA ACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCA ACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT	anti-ID B-2 light chain
77	ATGAGTGTGCTCACTCAGGTCCTGGCGTTGCTGCTGCTGTGGCT TACAGGTGCCAGATGT	anti-ID B-2 LC signal sequence
78	DYTFTSY	anti-ID HC-CDR1
79	DYTFTSYWMH	anti-ID HC-CDR1
80	TSYWMH	anti-ID HC-CDR1
81	YPGSGG	anti-ID HC-CDR2
82	NIYPGSGGTN	anti-ID HC-CDR2
83	WIGNIYPGSGGTN	anti-ID HC-CDR2
84	TREVTTVAYYYSM	anti-ID HC-CDR3
85	SNYLNWY	anti-ID LC-CDR1
86	LLIYYTSRLH	anti-ID LC-CDR2
87	QQGKTVPF	anti-ID LC-CDR3
88	GYTFTDY	anti-ID B-1 HC- CDR1

89	GYTFTDYMK	anti-ID B-1 HC-CDR1
90	TDYMK	anti-ID B-1 HC-CDR1
91	NPNNGG	anti-ID B-1 HC-CDR2
92	DINPNNGGTD	anti-ID B-1 HC-CDR2
93	WIGDINPNNGGTD	anti-ID B-1 HC-CDR2
94	AREGNNYGSRDAMD	anti-ID B-1 HC-CDR3
95	IYMYWY	anti-ID B-1 LC-CDR1
96	PWIYLTSLA	anti-ID B-1 LC-CDR2
97	QQWSSNPL	anti-ID B-1 LC-CDR3
98	GYSFTRY	anti-ID B-2 HC-CDR1
99	GYSFTRYWMN	anti-ID B-2 HC-CDR1
100	TRYWMN	anti-ID B-2 HC-CDR1
101	HPSDSE	anti-ID B-2 HC-CDR2
102	MIHPSDSETR	anti-ID B-2 HC-CDR2
103	WIGMIHPSDSETR	anti-ID B-2 HC-CDR2
104	ASIYYEE	anti-ID B-2 HC-CDR3
105	HNYLAWY	anti-ID B-2 LC-CDR1
106	LLVYNAKTLA	anti-ID B-2 LC-CDR2
107	QHFWSTPY	anti-ID B-2 LC-CDR3
108	GYX ₃ FX ₅ X ₆ YX ₈ MX ₁₀ X ₃ = T or S; X ₅ = T or S; X ₆ = D or R; X ₈ = Y or W; X ₁₀ = K or N	HC-CDR1 Consensus
109	WIGX ₄ IX ₆ PX ₈ X ₉ X ₁₀ X ₁₁ TX ₁₃ X ₁₄ NQX ₁₇ FKX ₂₀ X ₄ = D or M; X ₆ = N or H; X ₈ = N or S;	HC-CDR2 Consensus

	$X_9 = \text{N or D};$ $X_{10} = \text{G or S};$ $X_{11} = \text{G or E};$ $X_{13} = \text{D or R};$ $X_{14} = \text{Y or L};$ $X_{17} = \text{N or K};$ $X_{20} = \text{G or D}$	
110	$AX_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ $X_2 = \text{R or S};$ $X_3 = \text{E or I};$ $X_4 = \text{G or Y};$ $X_5 = \text{N or Y};$ $X_6 = \text{N or E};$ $X_7 = \text{Y or null};$ $X_8 = \text{G or null};$ $X_9 = \text{S or null};$ $X_{10} = \text{R or null};$ $X_{11} = \text{D or null};$ $X_{12} = \text{A or null};$ $X_{13} = \text{M or null};$ $X_{14} = \text{D or E};$ $X_{15} = \text{Y or A}$	HC-CDR3 Consensus
111	$X_1AX_3X_4X_5X_6X_7X_8YX_{10}X_{11}WY$ $X_1 = \text{S or R};$ $X_3 = \text{S or R};$ $X_4 = \text{S or G};$ $X_5 = \text{G or N};$ $X_6 = \text{V or I};$ $X_7 = \text{I or H};$ $X_8 = \text{N or null};$ $X_{10} = \text{M or L};$ $X_{11} = \text{Y or A}$	LC-CDR1 Consensus
112	$X_1X_2X_3YX_5X_6X_7X_8LAX_{11}$ $X_1 = \text{P or L};$ $X_2 = \text{W or L};$ $X_3 = \text{I or V};$ $X_5 = \text{L or N};$ $X_6 = \text{T or A};$ $X_7 = \text{S or K};$ $X_8 = \text{N or T};$ $X_{11} = \text{S or D}$	LC-CDR2 Consensus
113	$QX_2X_3X_4X_5X_6PX_8T$ $X_2 = \text{Q or H};$ $X_3 = \text{W or F};$ $X_4 = \text{S or W};$ $X_5 = \text{S or W};$ $X_6 = \text{N or T};$ $X_8 = \text{L or Y}$	LC-CDR3 Consensus
114	SYWMN	SJ25C1 HC-CDR1

115	QIYPGDGDTNYNGKFKG	SJ25C1 HC-CDR2
116	KTISSVVDFYFDY	SJ25C1 HC-CDR3
117	KASQNVGTNVA	SJ25C1 LC-CDR1
118	SATYRNS	SJ25C1 LC-CDR2
119	QQYNRYPYT	SJ25C1 LC-CDR3
120	DYGVS	FMC63 HC-CDR1
121	VIWGSETTYNSALKS	FMC63 HC-CDR2
122	HYYYGGSYAMDY	FMC63 HC-CDR3
123	RASQDISKYLN	FMC63 LC-CDR1
124	HTSRLHS	FMC63 LC-CDR2
125	QQGNTLPYT	FMC63 LC-CDR3
126	LEGGGEGRGSLTTCGDVEENPGPR	T2A
127	EGRGSLTTCGDVEENPGP	T2A
128	GSGATNFSLLKQAGDVEENPGP	P2A
129	ATNFSLLKQAGDVEENPGP	P2A
130	QCTNYALLKLAGDVESNPGP	E2A
131	VKQTLNFDLLKLAGDVESNPGP	F2A
132	QVQLKQSGPGLVQPSQSLITCTVS DYTFTSY GVHWVRQSPGKGLEWLGVI YPGSGG TDYNTPTFSTRSINKDNSKSQVFFKMNSLQSNDAIYYCAR EVTTVAYYYISMDY WGQGTLVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- SJ25C1 heavy chain
133	DILLTQSPVILSVSPGERVSFSC RASQDISNYLN WYQQRTNGSPRLIK YTSRLHS GIPSRFSGSGGTDFTLINSVESEDIADYYC QQGKTVPF FGAGTKLELKRTV AAPSVFIFPPSDEQLKSGTASVCLLNNFYFPREAKVQWKVDNALQ SGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	Exemplary humanized anti- SJ25C1 light chain
134	QVQLKQSGPGLVQPSQSLITCTVSGFSL TSYWMH WVRQSPGKGLE WIGNIYPGSGGTN YNTPTFSTRSINKDNSKSQVFFKMNSLQSNDAIYYC TREVTTVAYYYISMD YWGQGTLVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- SJ25C1 heavy chain

135	DILLTQSPVILSVSPGERVSFSCRASQSI SNYLNWY QQRTNGSPR LLIYYTSRLH SGIPSRFSGSGSGTDFTLSINSVESEDIADYYC QQGKTVPF TFGAGTKLELKRTV AAPSVFIFPP SDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Exemplary humanized anti- SJ25C1 light chain
136	QVQLKQSGPGLVQPSQSLITCTVSGFSLT SYWMH WVRQSPGKGLEWLG NIYPGSGGTNYDEKFKR RLSINKDNSKSQVFFKMNSLQSNDAIYYCAR EVTTVAYYYSDY WGQGLTVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- SJ25C1 heavy chain
137	DILLTQSPVILSVSPGERVSFSC RASQDISNYLN WYQQRTNGSPRLLIK YTSRLHS GIPSRFSGSGSGTDFTLSINSVESEDIADYYC QQGKTVPFT FGAGTKLELKRTV AAPSVFIFPP SDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Exemplary humanized anti- SJ25C1 light chain
138	QVQLKQSGPGLVQPSQSLITCTVS GYTFTDY GVHWVRQSPGKGLEWLGVI NPNNGG TDYNTPTFSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCAR EGNNYGSRDAMDY WGQGLTVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- FMC63 heavy chain
139	DILLTQSPVILSVSPGERVSFSC SASSGVIYMY WYQQRTNGSPRLLIK LTSNLAS GIPSRFSGSGSGTDFTLSINSVESEDIADYYC QQWSSNPLT FGAGTKLELKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	Exemplary humanized anti- FMC63 light chain
140	QVQLKQSGPGLVQPSQSLITCTVSGFSLT DYYMK WVRQSPGKGLEWLG DINPNNGGTDYNQNFKG RLSINKDNSKSQVFFKMNSLQSNDAIYYCAR EGNNYGSRDAMDY WGQGLTVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT	Exemplary humanized anti- FMC63 heavy chain

	SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	
141	DILLTQSPVILSVSPGERVSFSC SASSGVIYMY WYQQRNGSPRLLIK LTSNLAS GIPSRFSGSGSGTDFTLSINSVESEDIADYYC QQWSSNPLT FGAGTKLELKRTV AAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Exemplary humanized anti- FMC63 light chain
142	QVQLKQSGPGLVQPSQSLITCTVSGFSL TDYYMK WVRQSPGKGLE WIGDINPNNGGTD YNTPTSRSLINKDNSKSQVFFKMNSLQSNDAIYYC AREGNNYGSRDAMD YWGQGLTVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- FMC63 heavy chain
143	DILLTQSPVILSVSPGERVSFSCRASQSI IYMYWY QQRNGSPR PWIIYLTSLA SGIPSRFSGSGSGTDFTLSINSVESEDIADYYC QQWSSNPL TFGAGTKLELKRTV AAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Exemplary humanized anti- FMC63 light chain
144	QVQLKQSGPGLVQPSQSLITCTVS GYSFTRY GVHWVRQSPGKGLEWLGVI HPSDSE TDYNTPTSRSLINKDNSKSQVFFKMNSLQSNDAIYYCAR IYYEEA WGQGLTVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- FMC63 heavy chain
145	DILLTQSPVILSVSPGERVSFSC RASGNIHNYLA WYQQRNGSPRLLIK NAKTLAD GIPSRFSGSGSGTDFTLSINSVESEDIADYYC QHFWSTPYT FGAGTKLELKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ	Exemplary humanized anti- FMC63 light chain

	SGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	
146	QVQLKQSGPGLVQPSQSLITCTVSGFSLT RYWMN WVRQSPGKGLEWLG MIHPSDSETRLNQKFKD RLSINKDNSKSQVFFKMNSLQSNDAIYYCAR IYYEEA WGQGTLLVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- FMC63 heavy chain
147	DILLTQSPVILSVSPGERVSFSC RASGNIHNYLA WYQQRNGSPRLLIK NAKTLAD GIPSRFSGSGGTDFTLINSVESEDIADYYC QHFWSTPYT FGAGTKLELKRTV AAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Exemplary humanized anti- FMC63 light chain
148	QVQLKQSGPGLVQPSQSLITCTVSGFSL TRYWMN WVRQSPGKGLE WIGMIHPSDSETR YNTPTSRSLINKDNSKSQVFFKMNSLQSNDAIYYC ASIYYEE YWGQGTLLVTVSA TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	Exemplary humanized anti- FMC63 heavy chain
149	DILLTQSPVILSVSPGERVSFSCRASQSI HNYLAWY QQRNGSPR LLVYNKTLA SGIPSRFSGSGGTDFTLINSVESEDIADYYC QHFWSTPY TFGAGTKLELKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	Exemplary humanized anti- FMC63 light chain
150	ESKYGPPCPPCP	spacer (IgG4hinge) (aa) Homo sapiens
151	GAATCTAAGTACGACCGCCCTGCCCCCTTGCCCT	spacer (IgG4hinge) (nt) Homo sapiens
152	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSV MHEALHNHYTQKSLSLSPGK	Hinge-CH3 spacer Homo sapiens
153	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVD	Hinge-CH2-CH3

	VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSSFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS LSLGK	spacer Homo sapiens
154	RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEK KKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAVQDLWLRDKAT FTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSNGSQSQH SRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLS LNLLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAP ARPPQPQGSTTFWAWSVLRVPAPPSPQPATYTCVVSHEDSRTL N ASRSLEVS YVTDH	IgD-hinge-Fc Homo sapiens
155	RKVCNGIGIGEFKDSLSINATNIKHFNCTSSISGDLHILPVAFRGDS FTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIR GRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYAN TINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGP EPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVW KYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVG ALLLLL VVALGIGLFM	tEGFR artificial
156	MLLLVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHFNCTSSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLI QAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSL KEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGP EPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVW KYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVGALLLLL VVALGIGLFM	tEGFR artificial
157	FWVLVVVGVLACYSLLVTVAFIIFWV	CD28 (amino acids 153-179 of Accession No. P10747) Homo sapiens
158	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP FWVLVVVGVLACYSLLVTVAFIIFWV	CD28 (amino acids 114-179 of Accession No. P10747) Homo sapiens
159	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	CD28 (amino acids 180-220 of P10747) Homo sapiens
160	RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	CD28 (LL to GG) Homo sapiens
161	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	4-1BB (amino acids 214-255 of Q07011.1) Homo sapiens
162	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHD	CD3 zeta Homo sapiens

	GLYQGLSTATKDTYDALHMQALPPR	
163	RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR	CD3 zeta Homo sapiens
164	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR	CD3 zeta Homo sapiens

CLAIMS

1. An anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, or that contains variable regions derived from SJ25C1.
2. The anti-idiotypic antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises:
 - a light chain variable (VL) region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 5; and/or
 - a heavy chain variable (VH) region comprising at least 90% sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 1.
3. An antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment comprises:
 - a VL region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 5; and/or
 - a VH region comprising at least 90 % sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 1.
4. The anti-idiotypic antibody or antigen-binding fragment of claim 2 or claim 3, wherein:
 - the VH region comprises a heavy chain complementarity determining region 3 (CDR-H3) comprising the amino acid sequence set forth in SEQ ID NO: 11 or 84 or comprising a CDR-H3 contained within the VH sequence set forth in SEQ ID NO: 1; and/or
 - the VL region comprises a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence set forth in SEQ ID NO: 14 or 87 or comprising a CDR-L3 contained within the VL sequence set forth in SEQ ID NO: 5.
5. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 2-4, wherein:

the VH region comprises a CDR-H1 and a CDR-H2, respectively, comprising the amino acid sequences of CDR-H1 and CDR-H2 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 1; and/or

the VL region comprises a CDR-L1 and CDR-L2, respectively, comprising the amino acid sequences of CDR-L1 and CDR-L2 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 5.

6. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 2-5, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 9, 78, 79, or 80, a CDR-H2 set forth in SEQ ID NO: 10, 81, 82, or 83 and a CDR-H3 set forth in SEQ ID NO: 11 or 84; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 12 or 85, a CDR-L2 set forth in SEQ ID NO: 13 or 86, and a CDR-L3 set forth in SEQ ID NO: 14 or 87.

7. An anti-idiotypic antibody or antigen-binding fragment thereof comprising:
a CDR-H1, a CDR-H2, and a CDR-H3, respectively, comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 1; and/or

a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 5.

8. An anti-idiotypic antibody or antigen-binding fragment thereof comprising:
a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 9, 78, 79, or 80, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 10, 81, 82 or 83, and a CDR-H3 comprising the amino acid sequence set forth as SEQ ID NO: 11 or 84; and/or

a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 12 or 85, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 13 or 86, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 14 or 87.

9. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-8, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 1; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 5.

10. The anti-idiotypic antibody or antigen-binding fragment thereof of claim 9, wherein the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 1 and the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 5.

11. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-10, wherein the target antibody or antigen-binding fragment comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

12. An anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof.

13. The anti-idiotypic antibody or antigen-binding fragment of claim 12, wherein the antibody or antigen-binding fragment comprises:

a light chain variable (VL) region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62; and/or

a heavy chain variable (VH) region comprising at least 90% sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58.

14. An antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment comprises:

a VL region comprising at least 90% sequence identity to the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62; and/or

a VH region comprising at least 90 % sequence identity to the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58.

15. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-14, wherein:

the VH region comprises:

a heavy chain complementarity determining region 1 (CDR-H1) comprising the amino acid sequence of GYX₃FX₅X₆YX₈MX₁₀ (SEQ ID NO: 108), wherein X₃ is T or S, X₅ is T or S, X₆ is D or R, X₈ is Y or W, and X₁₀ is K or N;

a heavy chain complementarity determining region 2 (CDR-H2) comprising the amino acid sequence WIGX₄IX₆PX₈X₉X₁₀X₁₁TX₁₃X₁₄NQX₁₇FKX₂₀ (SEQ ID NO: 109), wherein X₄ is D or M, X₆ is N or H, X₈ is N or S, X₉ is N or D, X₁₀ is G or S, X₁₁ is G or E, X₁₃ is D or R, X₁₄ is Y or L, X₁₇ is N or K, and X₂₀ is G or D;

a heavy chain complementarity determining region 3 (CDR-H3) comprising the amino acid sequence AX₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅ (SEQ ID NO: 110), wherein X₂ is R or S, X₃ is E or I, X₄ is G or Y, X₅ is N or Y, X₆ is N or E, X₇ is Y or null, X₈ is G or null, X₉ is S or null, X₁₀ is R or null, X₁₁ is D or null, X₁₂ is A or null, X₁₃ is M or null, X₁₄ is D or E, and X₁₅ is Y or A; and/or

the VL region comprises:

a light chain complementarity determining region 3 (CDR-L1) comprising the amino acid sequence X₁AX₃X₄X₅X₆X₇X₈YX₁₀X₁₁WY (SEQ ID NO: 111), wherein X₁ is S or R, X₃ is S or R, X₄ is S or G, X₅ is G or N, X₆ is V or I, X₇ is I or H, X₈ is N or null, X₁₀ is M or L, and X₁₁ is Y or A;

a light chain complementarity determining region 2 (CDR-L2) comprising the amino acid sequence X₁X₂X₃YX₅X₆X₇X₈LAX₁₁ (SEQ ID NO: 112), wherein X₁ is P or L, X₂ is W or L, X₃ is I or V, X₅ is L or N, X₆ is T or A, X₇ is S or K, X₈ is N or T, and X₁₁ is S or D;

a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence QX₂X₃X₄X₅X₆PX₈T (SEQ ID NO: 113), wherein X₂ is Q or H, X₃ is W or F, X₄ is S or W, X₅ is S or W, X₆ is N or T, and X₈ is L or Y.

16. The anti-idiotypic antibody or antigen-binding fragment of claim 15, wherein:
the complementarity determining region 3 (CDR-H3) comprises the amino acid sequence set forth in SEQ ID NO: 94 or 104 or comprises a CDR-H3 contained within the VH sequence set forth in SEQ ID NO: 36 or 58; and/or

the light chain complementarity determining region 3 (CDR-L3) comprises the amino acid sequence set forth in SEQ ID NO: 97 or 107 or comprising a CDR-L3 contained within the VL sequence set forth in SEQ ID NO: 40 or 62.

17. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-16, wherein:

the VH region comprises a CDR-H1 and a CDR-H2, respectively, comprising the amino acid sequences of CDR-H1 and CDR-H2 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or

the VL region comprises a CDR-L1 and CDR-L2, respectively, comprising the amino acid sequences of CDR-L1 and CDR-L2 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62.

18. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-17, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 88, 89, 90, 98, 99, or 100, a CDR-H2 set forth in SEQ ID NO: 91, 92, 93, 101, 102, or 103 and a CDR-H3 set forth in SEQ ID NO: 94 or 104; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 95 or 105, a CDR-L2 set forth in SEQ ID NO: 96 or 106, and a CDR-L3 set forth in SEQ ID NO: 97 or 107.

19. An anti-idiotypic antibody or antigen-binding fragment thereof comprising:

a CDR-H1, a CDR-H2, and a CDR-H3, respectively, comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36 or 58; and/or

a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40 or 62.

20. An anti-idiotypic antibody or antigen-binding fragment thereof comprising:

a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 88, 89, 90, 98, 99, or 100, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 91, 92, 93, 101, 102, or 103, and a CDR-H3 comprising the amino acid sequence set forth as SEQ ID NO: 94 or 104; and/or

a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 95 or 105, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 96 or 106, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 97 or 107.

21. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 13-21, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36 or 58; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40 or 62.

22. The anti-idiotypic antibody or antigen-binding fragment thereof of claim 21, wherein the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36 or 58 and the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40 or 62.

23. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-22, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 44, 88, 89, or 90, a CDR-H2 set forth in SEQ ID NO: 45, 91, 92, or 93 and a CDR-H3 set forth in SEQ ID NO: 46 or 94; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 47 or 95, a CDR-L2 set forth in SEQ ID NO: 48 or 96, and a CDR-L3 set forth in SEQ ID NO: 49 or 97.

24. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-23, wherein:

the VH region comprises a CDR-H1 set forth in SEQ ID NO: 65, 98, 99, or 100, a CDR-H2 set forth in SEQ ID NO: 66, 101, 102, or 103 and a CDR-H3 set forth in SEQ ID NO: 67 or 104; and/or

the VL region comprises a CDR-L1 set forth in SEQ ID NO: 68 or 105, a CDR-L2 set forth in SEQ ID NO: 69 or 106, and a CDR-L3 set forth in SEQ ID NO: 100 or 107.

25. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-24, wherein:

the VH region comprises a CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 36; and/or

the VL region comprises a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 40.

26. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-24, wherein:

the VH region comprises a CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 sequences contained within the VH region amino acid sequence set forth in SEQ ID NO: 58; and/or

the VL region comprises a CDR-L1, a CDR-L2, and a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 sequences contained within the VL region amino acid sequence set forth in SEQ ID NO: 62.

27. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-25, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 36; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 40.

28. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 13-24 and 26, wherein:

the VH region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 58; and/or

the VL region of the antibody or fragment comprises the amino acid sequence of SEQ ID NO: 62.

29. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-28, wherein the target antibody or antigen-binding fragment is a single chain fragment.

30. The anti-idiotypic antibody or antigen-binding fragment of claim 29, wherein the fragment comprises antibody variable regions joined by a flexible linker.

31. The anti-idiotypic antibody or antigen-binding fragment of claim 29 or claim 30, wherein the fragment comprises an scFv.

32. The anti-idiotypic antibody or antigen-binding fragment of any of claims 1-11 and 29-31, wherein the target antibody or antigen-binding fragment:

comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24; and/or

is an scFv comprising the sequence of amino acids set forth in SEQ ID NO: 28.

33. The anti-idiotypic antibody or antigen-binding fragment of any of claims 12-31, wherein the target antibody or antigen-binding fragment:

comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31; and/or

is an scFv comprising the sequence of amino acids set forth in SEQ ID NO: 34.

34. An anti-idiotypic antibody or antigen-binding fragment thereof, wherein the anti-idiotypic antibody or antigen-binding fragment specifically binds to the same or an overlapping epitope of a target antibody or antigen-binding fragment thereof as the epitope specifically bound by the anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-33.

35. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-34, wherein:

the target antibody or antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR); and/or

the anti-idiotypic antibody or antigen-binding fragment specifically binds the target antibody or antigen-binding fragment comprised within or included in the antigen-binding domain of the extracellular portion of a CAR.

36. The anti-idiotypic antibody or antigen-binding fragment of claim 35, wherein the target antibody or antigen-binding fragment is an scFv and the anti-idiotypic antibody or antigen-binding fragment specifically binds to an epitope in the scFv of the CAR.

37. The anti-idiotypic antibody or antigen-binding fragment thereof of any of claims 1-11, 29-32 and 35, wherein the antibody or fragment specifically binds to a single chain variable fragment (scFv) derived from antibody SJ25C1 comprised in the extracellular portion

of a chimeric antigen receptor, optionally wherein the scFv derived from antibody SJ25C1 comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24; and/or comprises the sequence of amino acids set forth in SEQ ID NO: 28.

38. The anti-idiotypic antibody or antigen-binding fragment thereof of any of claims 12-31 and 33-35, wherein the antibody or fragment specifically binds to a single chain variable fragment (scFv) derived from antibody FMC63, or that contains variable regions derived from FMC63, comprised in the extracellular portion of a chimeric antigen receptor, optionally wherein the scFv derived from antibody FMC63 comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31; and/or comprises the sequence of amino acids set forth in SEQ ID NO: 34.

39. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-38, wherein the anti-idiotypic antibody or antigen-binding fragment specifically binds to an epitope within or including all or a portion of a complementarity determining region (CDR) of the target antibody or antigen-binding fragment.

40. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 35-39, wherein the CAR further comprises a transmembrane domain linked to the antigen-binding domain via a spacer.

41. The anti-idiotypic antibody of claim 40, wherein the spacer comprises an extracellular portion from CD28, which optionally is human CD28.

42. The anti-idiotypic antibody or antigen-binding fragment of claim 41, wherein the extracellular portion from CD28 comprises the sequence of amino acids set forth in SEQ ID NO: 27.

43. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 40-42, wherein the transmembrane domain comprises a transmembrane portion of CD28, which optionally is human CD28.

44. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 40-43, wherein the antibody or fragment does not bind to an epitope in the spacer domain of the CAR.

45. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-44, wherein the antibody or fragment does not bind or does not specifically bind to CD28 or a portion thereof, which optionally is human CD28, which optionally comprises an extracellular portion of CD28, which optionally comprises the sequence of amino acids set forth in SEQ ID NO: 27.

46. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-45, wherein the antibody or fragment does not bind to an epitope in an Fc domain, which optionally is a human IgG1 Fc domain.

47. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-46, wherein the target antibody or antigen-binding fragment specifically binds to human CD19.

48. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-47, wherein the anti-idiotypic antibody or fragment does not cross-react with another anti-CD19 antibody, which optionally is comprised in the extracellular antigen-binding domain of another CAR.

49. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-48, wherein the anti-idiotypic antibody or fragment does not cross-react with another CAR.

50. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-49, wherein the anti-idiotypic antibody or fragment is an agonist antibody of a CAR comprising the target antibody or antigen-binding fragment.

51. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-49, wherein the antibody or fragment is an antagonist of a CAR comprising the target antibody or antigen-binding fragment.

52. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-51, which is humanized.

53. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-52, which is recombinant.

54. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-53, which is monoclonal.

55. The anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-54, which is an antigen-binding fragment.

56. The anti-idiotypic antibody or antigen-binding fragment of claim 55, wherein the antigen-binding fragment is selected from among fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, a single chain variable fragment (scFv) or a single domain antibody.

57. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-54, comprising at least a portion of an immunoglobulin constant region.

58. The anti-idiotypic antibody or antigen-binding fragment of claim 57, wherein the at least a portion of an immunoglobulin constant region comprises an Fc region or a portion of the Fc comprising the CH2 and CH3 domains.

59. The anti-idiotypic antibody or antigen-binding fragment of claim 57 or claim 58, wherein the constant region is derived from human IgG.

60. The anti-idiotypic antibody or antigen-binding fragment of any one of claims 57-59, which is an intact antibody or full-length antibody.

61. A conjugate, comprising the anti-idiotypic antibody or antigen-binding fragment of any one of claims 1-60 and a heterologous molecule or moiety.

62. The conjugate of claim 61, wherein the heterologous molecule or moiety is a label.

63. The conjugate of claim 62, wherein the label is selected from a fluorescent dye, a fluorescent protein, a radioisotope, a chromophore, a metal ion, a gold particle, a silver particle, a magnetic particle, a polypeptide, an enzyme, a streptavidin, a biotin, a luminescent compound or an oligonucleotide.

64. The conjugate of claim 62, wherein the heterologous molecule or moiety is a protein, peptide, nucleic acid or small molecule, which optionally is or comprises a toxin or a Strep-Tag.

65. A nucleic acid molecule(s) encoding the heavy chain and/or light chain of the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-60.

66. The nucleic acid molecule of claim 65, comprising:

a sequence of nucleotides encoding (i) the heavy chain variable region set forth in SEQ ID NO: 15, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 15; or (iii) a degenerate sequence of (i) or (ii); and/or
a sequence of nucleotides encoding (iv) the light chain variable region set forth in SEQ ID NO: 19, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 19; or (vi) a degenerate sequence of (iv) or (v).

67. The nucleic acid molecule of claim 65 or claim 66, comprising:

a sequence of nucleotides encoding (i) the heavy chain set forth in SEQ ID NO: 17, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 17; or (iii) a degenerate sequence of (i) or (ii); and/or
a sequence of nucleotides encoding (iv) the light chain set forth in SEQ ID NO: 21, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 21; or (vi) a degenerate sequence of (iv) or (v).

68. The nucleic acid molecule of claim 65, comprising:

a sequence of nucleotides encoding (i) the heavy chain variable region set forth in SEQ ID NO: 50 or 71, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 50 or 71; or (iii) a degenerate sequence of (i) or (ii); and/or
a sequence of nucleotides encoding (iv) the light chain variable region set forth in SEQ ID NO: 54 or 75, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 54 or 75; or (vi) a degenerate sequence of (iv) or (v).

69. The nucleic acid molecule of claim 65 or claim 68, comprising:

a sequence of nucleotides encoding (i) the heavy chain set forth in SEQ ID NO: 52 or 73, (ii) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 52 or 73; or (iii) a degenerate sequence of (i) or (ii); and/or

a sequence of nucleotides encoding (iv) the light chain set forth in SEQ ID NO: 56 or 76, (v) a sequence of nucleotides that has at least 90% sequence identity to the sequence of nucleotides set forth in SEQ ID NO: 56 or 76; or (vi) a degenerate sequence of (iv) or (v).

70. The nucleic acid molecule of any one of claims 65-69, wherein the nucleotide sequence encoding the heavy chain and/or light chain comprises a signal sequence.

71. A vector, comprising the nucleic acid molecule of any one of claims 65-70.

72. A cell, comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-41 or the nucleic acid molecule of any one of claims 65-70.

73. A method of producing an anti-idiotypic antibody or antigen-binding fragment thereof, comprising expressing the heavy and/or light chain encoded by the nucleic acid molecule of any one of claims 65-70 or the vector of claim 71 in a suitable host cell and recovering or isolating the antibody.

74. A method of producing an anti-idiotypic antibody or antigen-binding fragment thereof, comprising culturing the cell of claim 72 under conditions in which the heavy chain and/or light chain is expressed and recovering or isolating the antibody.

75. An anti-idiotypic antibody or antigen-binding fragment thereof produced by the method of claim 73 or claim 74.

76. A composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-60, conjugate of any one of claims 61-64, or the cell of claim 72.

77. The composition of claim 76, further comprising a pharmaceutically acceptable excipient.

78. A kit, comprising one or more of the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-60, the conjugate of any one of claims 61-64, the nucleic acid of any one of claims 65-70, and, optionally, instructions for use.

79. The kit of claim 78, further comprising a reagent or support for immobilizing the anti-idiotypic antibody or antigen-binding fragment thereof or conjugate, wherein said reagent or support is a bead, a column, a microwell, a stick, a filter, a strip or a soluble oligomeric streptavidin mutein reagent.

80. A method of detecting a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment.

81. A method of detecting a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and

(b) detecting the anti-idiotypic antibody bound to the target antibody or antigen-binding fragment.

82. The method of claim 81, wherein the target antibody or antigen-binding fragment is bound to a cell or expressed on the surface of a cell and detecting in (b) comprises detecting cells bound with the anti-idiotypic antibody.

83. The method of claim 82, wherein the cell expresses on its surface a CAR comprising the target antibody or antigen-binding fragment.

84. A method of detecting a CAR comprising a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) detecting cells bound with the anti-idiotypic antibody.

85. A method of detecting a CAR comprising a target antibody or antigen-binding fragment thereof, comprising:

(a) contacting a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and

(b) detecting cells bound with the anti-idiotypic antibody.

86. The method of any one of claims 80-85, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is directly or indirectly labeled for detection.

87. A method of selecting cells from a cell population, comprising:

(a) contacting a cell population expressing a chimeric antigen receptor (CAR) comprising a target antibody or a cell bound to a target antibody with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, wherein the target antibody is the antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) selecting cells bound with the anti-idiotypic antibody.

88. A method of selecting cells from a cell population, comprising:

(a) contacting a cell population expressing a chimeric antigen receptor (CAR) comprising a target antibody or a cell bound to a target antibody with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, wherein the target antibody is the antibody FMC63 or an antigen-binding fragment thereof; and

(b) selecting cells bound with the anti-idiotypic antibody.

89. The method of claim 87 or claim 88, wherein the cells bound with the anti-idiotypic antibody are selected by affinity-based separation.

90. The method of claim 89, wherein the affinity-based separation is immunoaffinity-based separation.

91. The method of claim 89 or claim 90, wherein the affinity-based separation is by flow cytometry.

92. The method of claim 89 or claim 90, wherein the affinity-based separation is by magnetic activated cell sorting.

93. The method of claim 89 or claim 90, wherein the affinity-based separation comprises affinity chromatography.

94. The method of claim 92 or claim 93, wherein the anti-idiotypic antibody is reversibly bound or immobilized to a support or a stationary phase.

95. A method of stimulating cells, comprising incubating an input composition comprising cells expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, thereby generating an output composition comprising stimulated cells.

96. A method of stimulating cells, comprising incubating an input composition comprising cells expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, thereby generating an output composition comprising stimulated cells.

97. A method of producing a cell composition, comprising:

- (a) introducing into cells a nucleic acid molecule encoding a chimeric antigen receptor (CAR), thereby generating an input composition; and
- (b) incubating the input composition with an anti-idiotypic antibody or antigen-binding fragment thereof specific for the antigen receptor of the CAR, thereby producing the cell composition.

98. The method of claim 97, wherein the CAR comprises a target antibody that specifically binds to CD19.

99. The method of claim 98, wherein the target antibody is the antibody SJ25C1 or an antigen-binding fragment thereof.

100. The method of any of claims 97-99, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof.

101. The method of claim 98, wherein the target antibody is the antibody FMC63 or an antigen-binding fragment thereof.

102. The method of any of claims 97-98 and 101, wherein the anti-idiotypic antibody or antigen-binding fragment thereof specifically binds to a target antibody that is antibody FMC63 of any one of claims 12-31, 33-36 and 38-60 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof.

103. The method of any one of claims 97-102, wherein the introducing in (a) comprises introducing the nucleic acid molecule into the cells by viral transduction, transposition, electroporation, or chemical transfection.

104. The method of any one of claims 97-103, wherein the introducing in (a) comprises introducing the nucleic acid molecule in the cells by transduction with a viral vector comprising the nucleic acid molecule, optionally wherein the viral vector is a retroviral vector or a lentiviral vector.

105. The method of any of claims 97-103, wherein the introducing in (a) comprises introducing the nucleic acid molecule in the cells by transposition with a transposon comprising the nucleic acid molecule.

106. The method of any of claims 97-103, wherein the introducing in (a) comprises introducing the nucleic acid molecule in the cells by electroporation or transfection of a vector comprising the nucleic acid molecule.

107. The method of any one of claims 97-106, further comprising a step of activating the cells prior to step (a).

108. The method of claim 107, wherein the step of activating the cells comprises contacting the cells with an agonist of CD3 and optionally an agonist of CD28.

109. The method of claim 108, wherein the step of activating the cells comprises contacting the cells with a reagent comprising agonistic anti-CD3 and anti-CD28 antibodies.

110. The method of any one of claims 95-109, wherein the incubation is performed under conditions in which the anti-idiotypic antibody or antigen-binding fragment thereof binds to the CAR, thereby inducing or modulating a signal in one or more cells in the input composition.

111. The method of any one of claims 95-110, wherein the cells comprise T cells.

112. The method of claim 111, wherein the T cells comprise CD4+ and/or CD8+ T cells.

113. The method of any one of claims 95-112, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a solid support, which optionally comprises

or is conjugated to a reagent comprising a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof.

114. The method of any one of claims 95-112, wherein the anti-idiotypic antibody or antigen-binding fragment thereof is immobilized to a soluble reagent, which optionally is or comprises a plurality of binding sites capable of reversibly binding to the anti-idiotypic antibody or antigen-binding fragment thereof.

115. The method of claim 113 or claim 114, wherein the reagent comprises a streptavidin mutein.

116. The method of any one of claims 95-115, wherein the incubation is for at least or about at least 5 minutes, 10 minutes, 30 minutes, 60 minutes, 2 hours, 6 hours, 12 hours, 24 hours, 36, 48 hours, 72 hours or 96 hours.

117. The method of any one of claims 95-116, wherein the input composition comprises less than or less than about 60%, less than or less than about 50%, less than or less than about 40%, less than or less than about 30%, less than or less than about 20% or less than or less than about 10% CAR-expressing cells as a percentage of the total cells in the composition.

118. The method of any one of claims 95-117, wherein:
the number of CAR-expressing cells in the output composition is increased by greater than 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold or more compared to the number of CAR-expressing cells in the input composition; and/or
the percentage of CAR-expressing in the output composition compared to the total cells in the composition is increased by greater than 10 %, 20 %, 40 %, 50 %, 60 %, 70 %, 80 % or more.

119. The method of any one of claims 95-118, wherein prior to the introducing and/or incubating the cells are not selected or enriched for CAR-expressing cells.

120. The method of any one of claims 80, 82-84, 86, 87, 89-95, 97-100, 103-119, wherein the target antibody or antigen-binding fragment comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

121. The method of any one of claims 81, 82, 83, 85, 86, 88-94, 96-99, 101 and 102-119, wherein the target antibody or antigen-binding fragment comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

122. A method of purifying an antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof; and

(b) isolating complexes comprising the anti-idiotypic antibody.

123. A method of purifying an antibody or antigen-binding fragment thereof, comprising:

(a) contacting a composition comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof with the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or the conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof; and

(b) isolating complexes comprising the anti-idiotypic antibody.

124. The method of claim 122 or claim 123, wherein the complexes comprising the anti-idiotypic antibody are isolated by affinity-based separation.

125. The method of claim 124, wherein the affinity-based separation is immunoaffinity-based separation.

126. The method of claim 124, wherein the affinity-based separation is magnetic-based separation.

127. The method of claim 124, wherein the affinity-based separation comprises affinity chromatography.

128. A method of identifying an anti-idiotypic antibody or antigen-binding fragment, comprising:

(a) introducing into a subject a soluble immunization reagent comprising an antigen-binding fragment of a target antibody fused to a solubilizing moiety; and

(b) identifying an antibody from the subject that specifically binds to the target antibody or the antigen-binding fragment thereof.

129. The method of claim 128, wherein the antigen-binding fragment comprises the variable heavy chain region and/or variable light chain region of the target antibody.

130. The method of claims 128 or claim 129, wherein the antigen-binding fragment is a single chain fragment.

131. The method of claim 130, wherein the antigen-binding fragment is an scFv.

132. The method of any of claims 128-131, wherein the antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR).

133. The method of any of claims 128-132, wherein the solubilizing moiety is an Fc domain or fragment thereof, which optionally is a human IgG1 Fc.

134. The method of claim 133, wherein the solubilizing moiety is an Fc domain lacking the hinge region.

135. The method of claim 134, wherein the solubilizing moiety comprises the amino acid sequence set forth in SEQ ID NO: 32.

136. The method of any of claims 128-135, wherein identifying the antibody comprises:

(i) isolating B cells from the spleen of the subject and fusing them with immortalized B cells to generate hybridomas;

(ii) screening the hybridomas for production of antibodies that specifically bind the target antibody or the antigen-binding fragment thereof or a chimeric antigen receptor comprising the antigen-binding fragment; and

(iii) sequencing an antibody from a hybridoma producing an antibody that specifically binds, thereby identifying the anti-idiotypic antibody.

137. The method of any of claims 128-136, wherein the target antibody binds to CD19.

138. The method of any of claims 128-137, wherein the antigen-binding fragment of the target antibody is derived from antibody SJ25C1, optionally wherein the antigen-binding fragment of the target antibody comprises a heavy chain variable region set forth in SEQ ID NO: 23 and/or a light chain variable region set forth in SEQ ID NO: 24.

139. The method of any of claims 128-138, wherein the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody SJ25C1, optionally wherein the scFv comprises the sequence of amino acids set forth in SEQ ID NO:28.

140. The method of any of claims 128-137, wherein the antigen-binding fragment of the target antibody is derived from antibody FMC63, optionally wherein the antigen-binding fragment of the target antibody comprises a heavy chain variable region set forth in SEQ ID NO: 30 and/or a light chain variable region set forth in SEQ ID NO: 31.

141. The method of any of claims 128-137 and 140, wherein the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody FMC63, optionally wherein the scFv comprises the sequence of amino acids set forth in SEQ ID NO: 34.

142. A method of depleting cells, comprising administering, to a subject, a composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof.

143. A method of depleting cells, comprising administering, to a subject, a composition comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or conjugate of any one of claims 61-64 that specifically binds to a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof.

144. The method of claim 137 or claim 138, wherein the depletion occurs via antibody-dependent cell-mediated cytotoxicity (ADCC).

145. A method of determining the presence or absence of a molecule that binds to a chimeric antigen receptor (CAR), the method comprising:

(a) contacting a binding reagent with a sample from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof under conditions to form a complex comprising the binding reagent and a molecule from the sample that binds to the binding reagent, wherein the binding reagent comprises the extracellular domain of the CAR or a portion thereof comprising the target antibody or the antigen-binding fragment thereof; and

(b) detecting the presence or absence of the complex, thereby determining the presence or absence of a molecule that binds the CAR.

146. The method of claim 145, further comprising carrying out steps (a) and (b) on a positive control sample and, optionally, determining the presence or absence of the molecule by comparison to the positive control, wherein the positive control sample comprises the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or conjugate of any one of claims 61-64 that specifically binds to the target antibody or an antigen-binding fragment thereof.

147. A method of determining the presence or absence of a molecule that binds to a chimeric antigen receptor (CAR), the method comprising:

(a) contacting a binding reagent with a sample from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof under conditions to form a complex comprising the binding reagent and a molecule from the sample that binds to the binding reagent, wherein the binding reagent comprises the extracellular domain of the CAR or a portion of the extracellular domain comprising the target antibody or the antigen-binding fragment thereof;

(b) detecting the presence or absence of the complex.

148. The method of claim 147, further comprising carrying out steps (a) and (b) on a positive control sample and, optionally, determining the presence or absence of the molecule by comparison to the positive control, wherein the positive control sample comprises the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or conjugate of any one of claims 61-64 that specifically binds to the target antibody or an antigen-binding fragment thereof.

149. The method of any of claims 145-148, wherein the molecule that binds to the binding reagent is or comprises an antibody.

150. The method of any of claims 145-149, wherein the binding reagent is detectably labeled or is capable of producing a detectable signal.

151. The method of any of claims 145-150, wherein the binding reagent is bound to a solid support or is soluble.

152. The method of any of claims 145-153, wherein the complex is detected by an immunoassay.

153. The method of claim 152, wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA), chemiluminescent assay, electrochemiluminescent assay, surface plasmon resonance (SPR)-based biosensor (e.g., BIAcore), flow cytometry, or Western blot.

154. The method of claim 152 or claim 153, wherein the immunoassay comprises meso scale discovery.

155. The method of any of claims 152-154, wherein the immunoassay is a sandwich assay or bridge assay.

156. The method of any of claims 145-155, wherein the binding reagent is a first binding reagent and detecting the presence or absence of the complex comprises:

- (i) contacting the complex formed in step (a) with a second binding reagent, wherein the second binding reagent (1) comprises the extracellular domain of the CAR or a portion thereof comprising the target antibody or the antigen-binding fragment thereof, and (2) is detectably labeled or is capable of producing a detectable signal; and
- (ii) assessing the presence or absence of the detectable signal.

157. The method of claim 156, wherein:

the first binding reagent is bound to a solid support, optionally wherein first binding reagent is linked, directly or indirectly, to a biotin and/or bound to a solid support through a streptavidin; and/or

the second binding reagent is soluble.

158. The method of claim 156 or claim 157, wherein the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

159. The method of any of claims 150-158, wherein:

the detectable label is or comprises a fluorescent label, a chemiluminescent label, an electroluminescent label, a colorimetric label, a bioluminescent label or a radiolabel; and/or

the detectable signal is or comprises a fluorescent signal, chemiluminescent signal, electroluminescent signal, colorimetric signal, a bioluminescent signal or a radioactive signal.

160. The method of any of claims 150-159, wherein the detectable label is or comprises a SULFO-TAG.

161. The method of any of claims 145-160, wherein the antigen-binding fragment of the target antibody comprises the variable heavy chain region and/or variable light chain region of the target antibody.

162. The method of claim 145-161, wherein the antigen-binding fragment of the target antibody is a single chain fragment.

163. The method of any of claims 145-162, wherein the antigen-binding fragment of the target antibody is an scFv.

164. The method of any of claims 145-163, wherein the sample comprises whole blood, serum or plasma.

165. An article of manufacture comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of claims 61-64, and instructions for using the anti-idiotypic antibody to detect an SJ25C1 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof; to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody SJ25C1 or an antigen-binding fragment thereof; to stimulate an input composition comprising cells expressing a chimeric antigen receptor comprising the SJ25C1 antibody or antigen-binding fragment thereof.

166. An article of manufacture comprising the anti-idiotypic antibody or antigen-binding fragment thereof of any one of claims 12-31, 33-36 and 38-60 or the conjugate of any one of claims 61-64, and instructions for using the anti-idiotypic antibody to detect an FMC63 antibody or antigen-binding fragment thereof or a chimeric antigen receptor comprising the FMC63 antibody or antigen-binding fragment thereof; to select or enrich, from a population of cells, engineered cells expressing a chimeric antigen receptor (CAR) comprising the antibody FMC63 or an antigen-binding fragment thereof; to stimulate an input composition comprising

cells expressing a chimeric antigen receptor comprising the FMC63 antibody or antigen-binding fragment thereof.

167. An article of manufacture comprising:

a binding reagent comprising the extracellular domain of a chimeric antigen receptor (CAR) comprising a target antibody that is antibody FMC63 or an antigen-binding fragment thereof, said extracellular domain or portion thereof comprising the target antibody or antigen-binding fragment thereof; and

an anti-idiotypic antibody or antigen-binding fragment of any of claims 12-31, 33-36 and 38-60 or the conjugate of any one of claims 61-64.

168. The article of manufacture of claim 167, wherein the binding reagent is a first binding reagent and the article of manufacture further comprises a second binding reagent comprising the extracellular domain or portion thereof of the CAR.

169. The article of manufacture of claim 167 or claim 168, wherein the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

170. The article of manufacture of any of claims 167-169, further comprising instructions for using the binding reagent, optionally the first and second binding reagent, for assaying a sample for the presence or absence of a molecule that binds to the binding reagent using an immunoassay, optionally wherein the immunoassay is a bridge or sandwich immunoassay, optionally wherein the sample is from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody FMC63 or an antigen-binding fragment thereof.

171. An article of manufacture comprising:

a binding reagent comprising the extracellular domain of a chimeric antigen receptor (CAR) comprising a target antibody that is antibody SJ25C1 or an antigen-binding fragment thereof, said extracellular domain or portion thereof comprising the target antibody or antigen-binding fragment thereof; and

an anti-idiotypic antibody or antigen-binding fragment of any of claims 1-11 and 29-32, 34-37 and 39-60 or the conjugate of any one of claims 61-64.

172. The article of manufacture of claim 171, wherein the binding reagent is a first binding reagent and the article of manufacture further comprises a second binding reagent comprising the extracellular domain or portion thereof of the CAR.

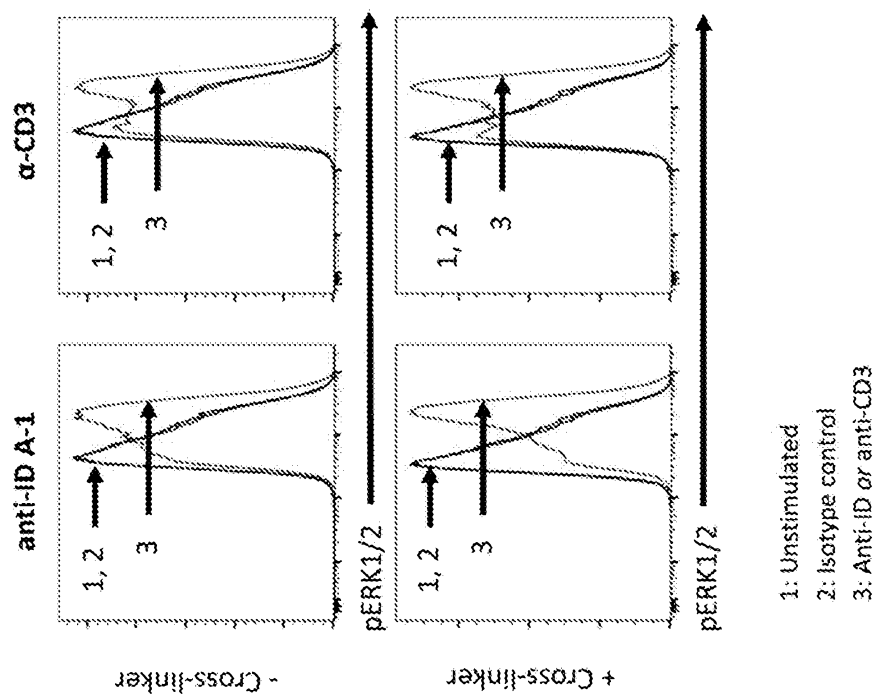
173. The article of manufacture of claim 171 or claim 172, wherein the extracellular domain of the CAR or portion thereof of the first and second binding reagent is the same.

174. The article of manufacture of any of claims 171-173, further comprising instructions for using the binding reagent, optionally the first and second binding reagent, for assaying a sample for the presence or absence of a molecule that binds to the binding reagent using an immunoassay, optionally wherein the immunoassay is a bridge or sandwich immunoassay, optionally wherein the sample is from a subject having been administered a cell therapy comprising cells engineered with a CAR comprising a target antibody that is the antibody SJ25C1 or an antigen-binding fragment thereof.

175. The article of manufacture of any of claims 167-174, wherein the binding reagent, optionally the first and/or second binding reagent, is detectably labeled or capable of producing a detectable signal.

176. The article of manufacture of any of claims 168-170 and 172-175, wherein one of the first and second binding reagent is attached to a solid support or is capable of being attached to a solid support and the other of the first and second binding reagent is detectable label or is capable of producing a detectable signal.

177. The method of claim 176, wherein the article of manufacture further comprises a solid support, optionally wherein the one of the first and second binding reagent is linked, directly or indirectly to biotin, and the solid support comprises a streptavidin-coated surface.

FIG. 1

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FIG. 2A

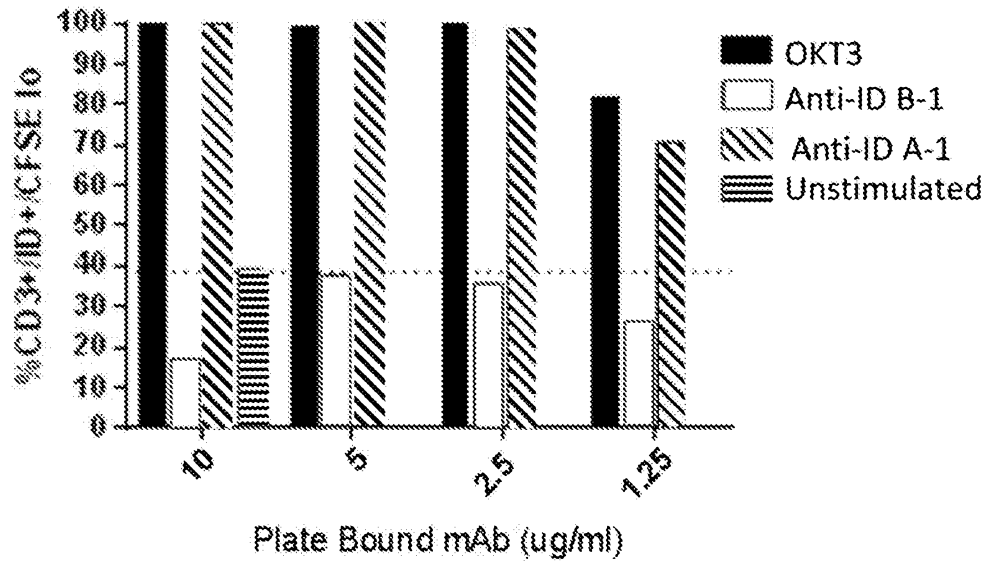
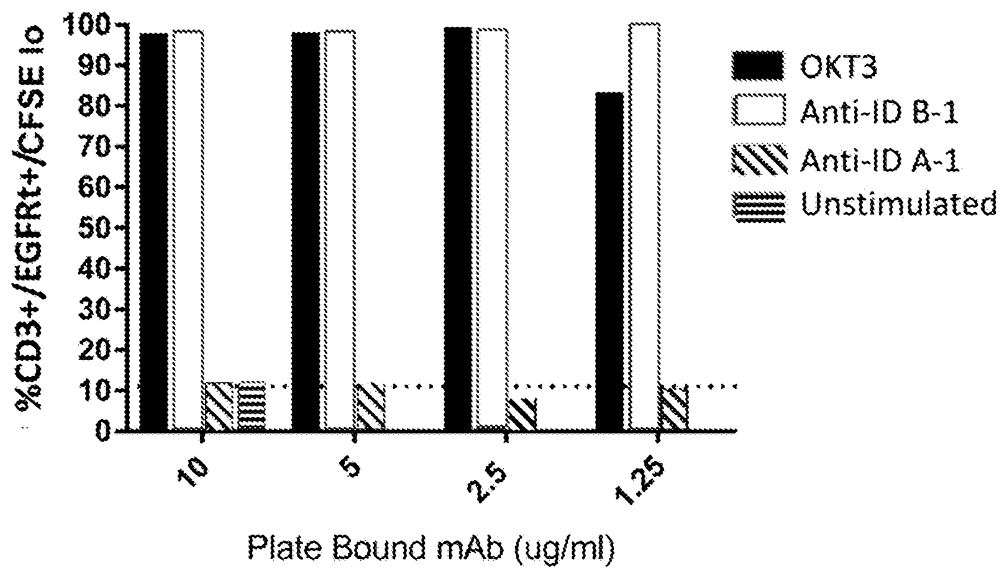


FIG. 2B



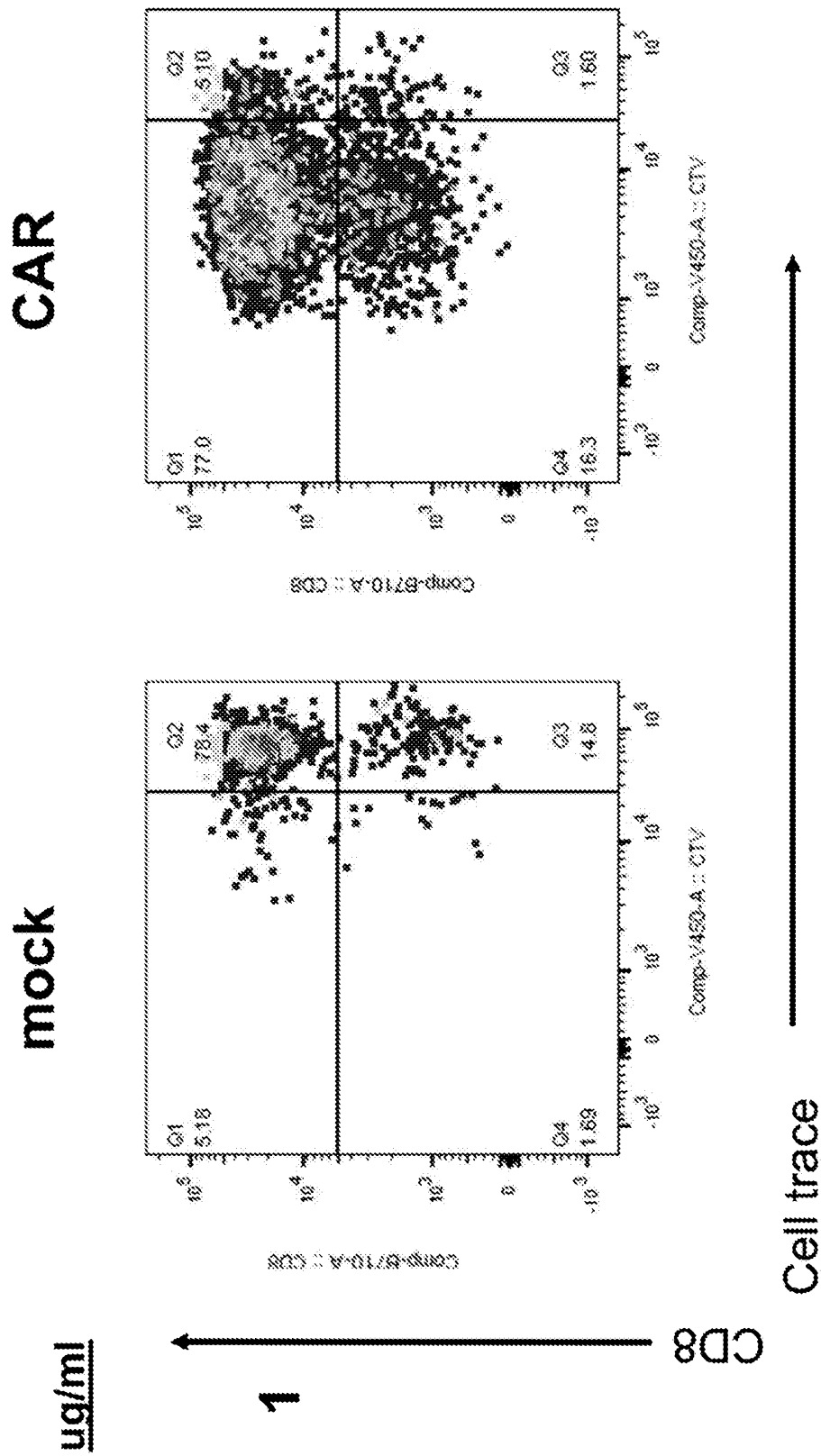


FIG. 2C

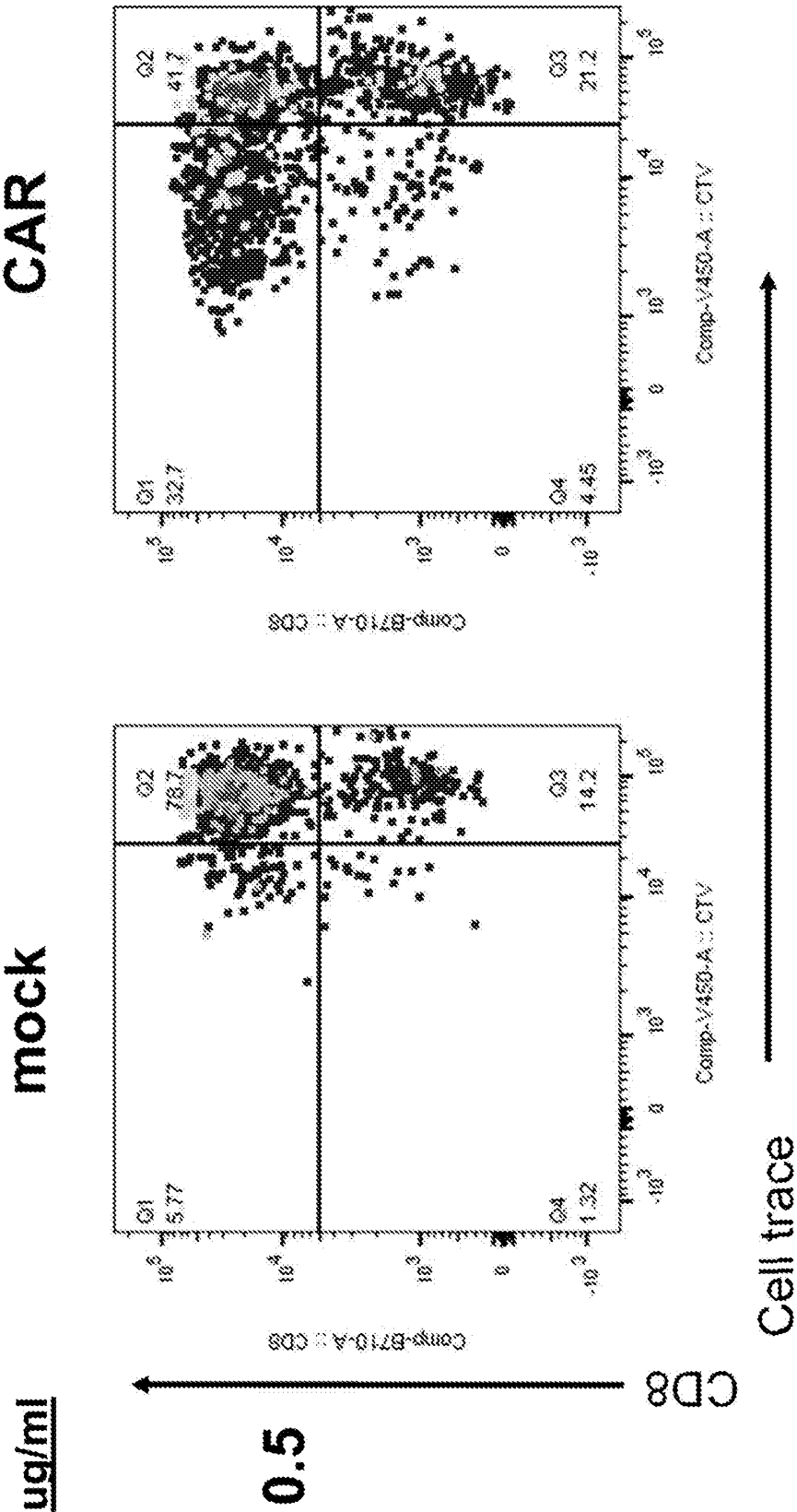


FIG. 2C Cont.

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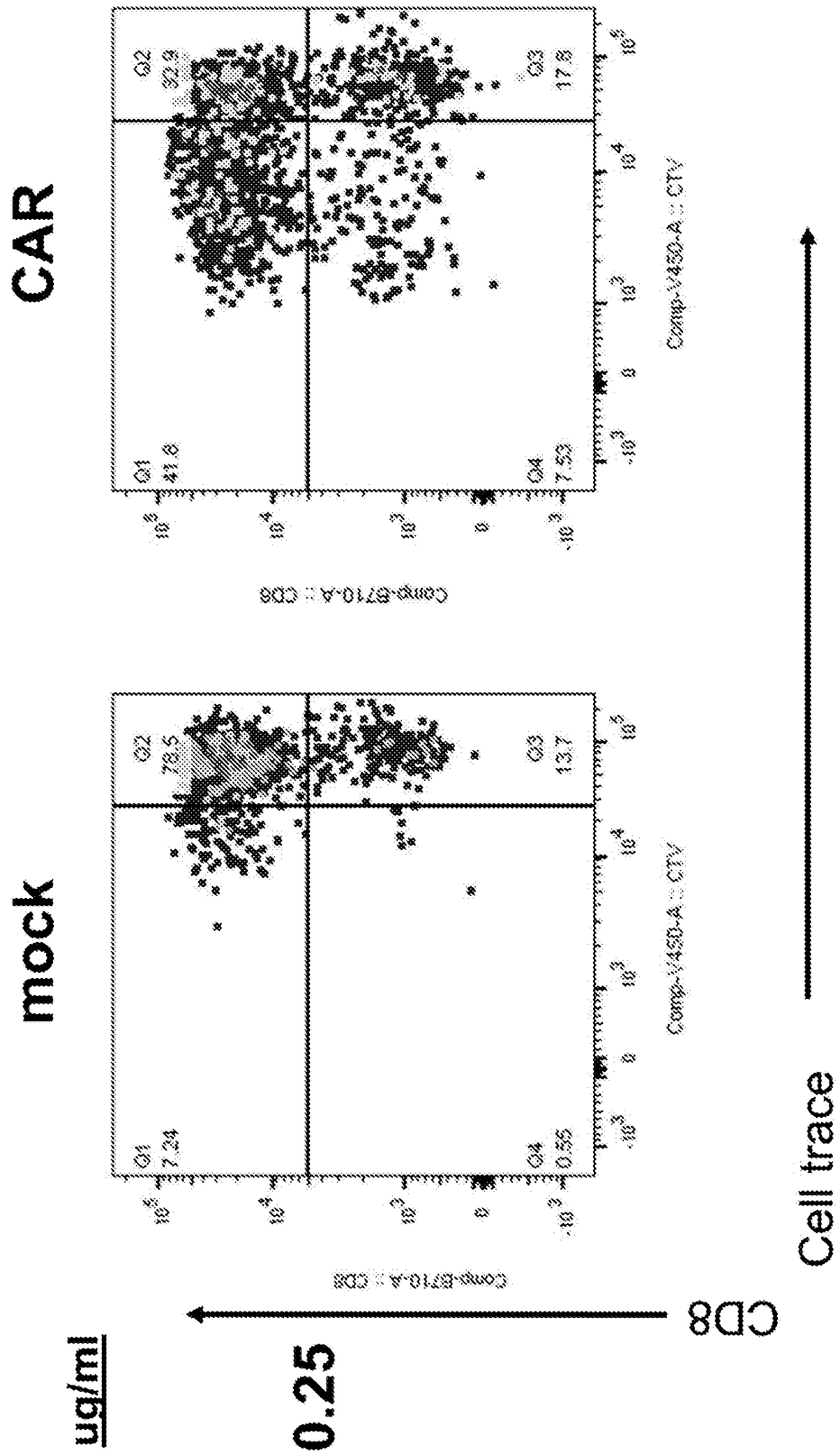
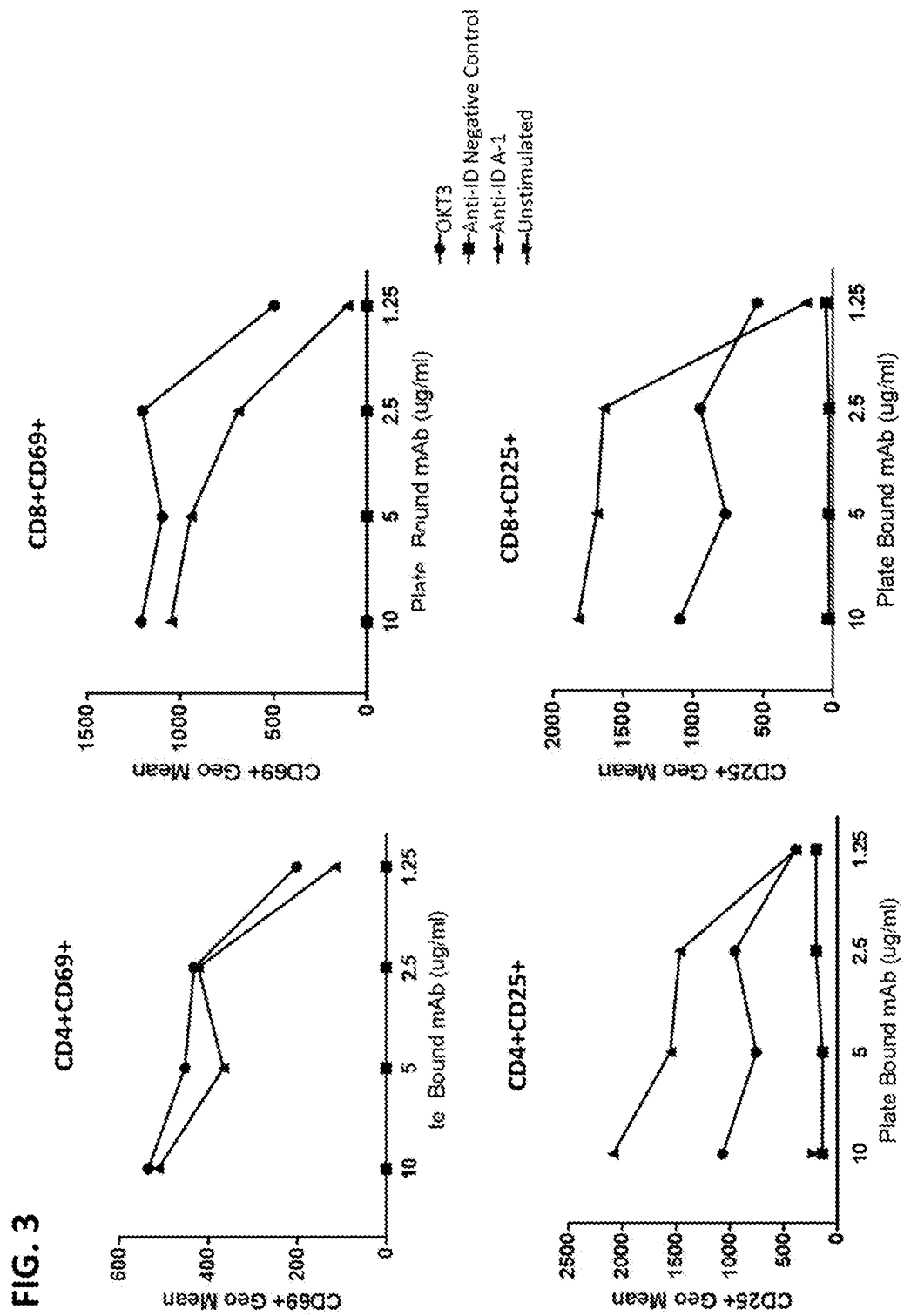
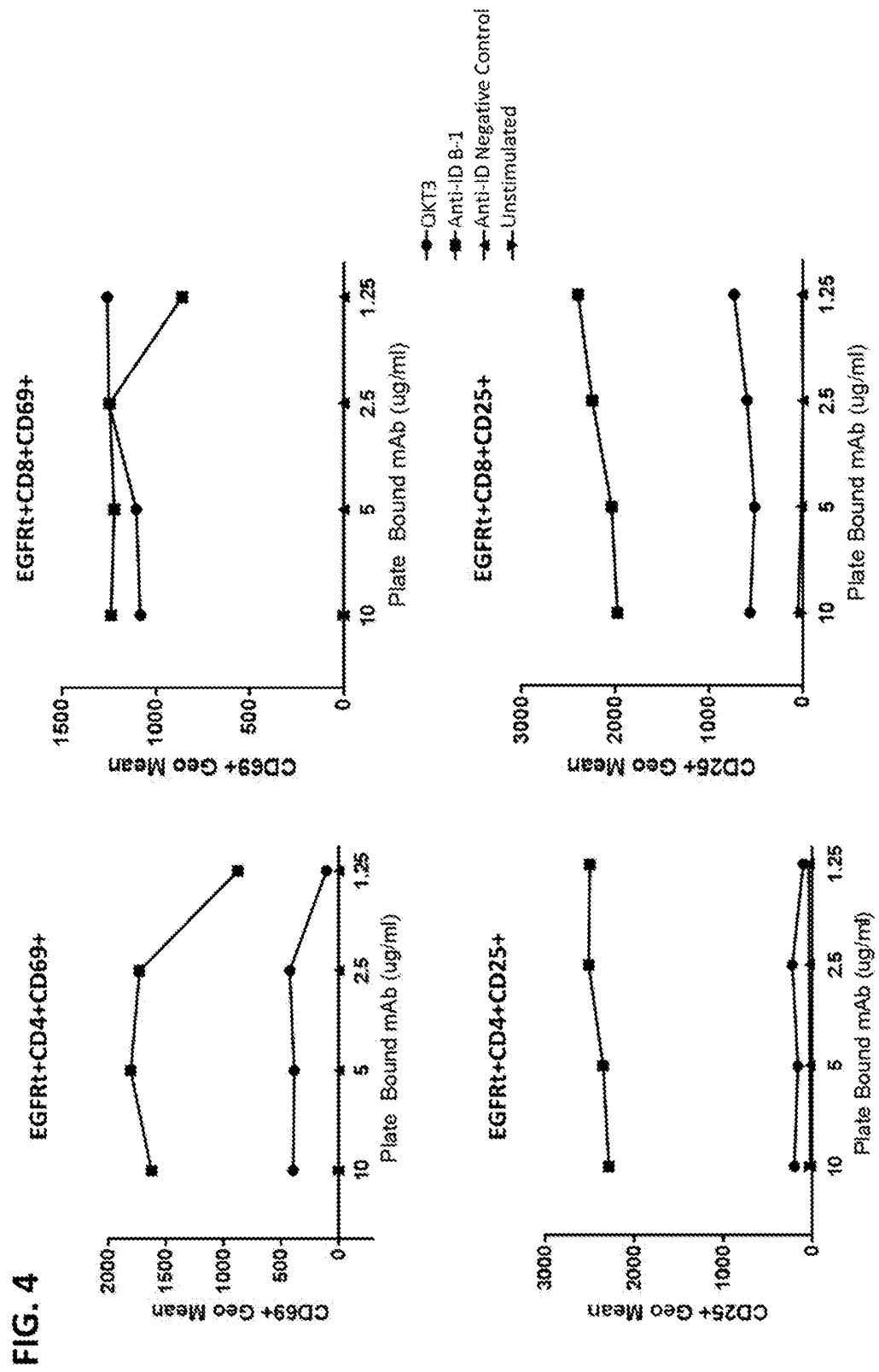


FIG. 2C Cont.

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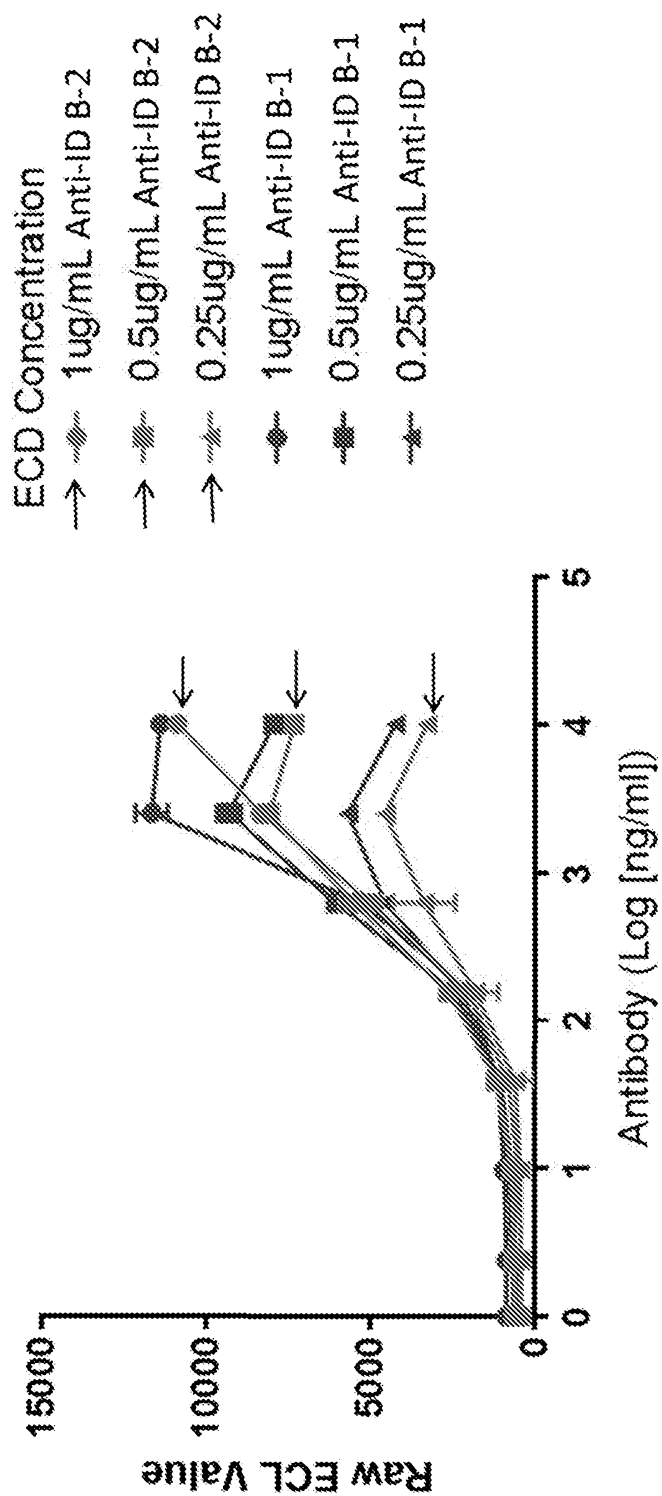


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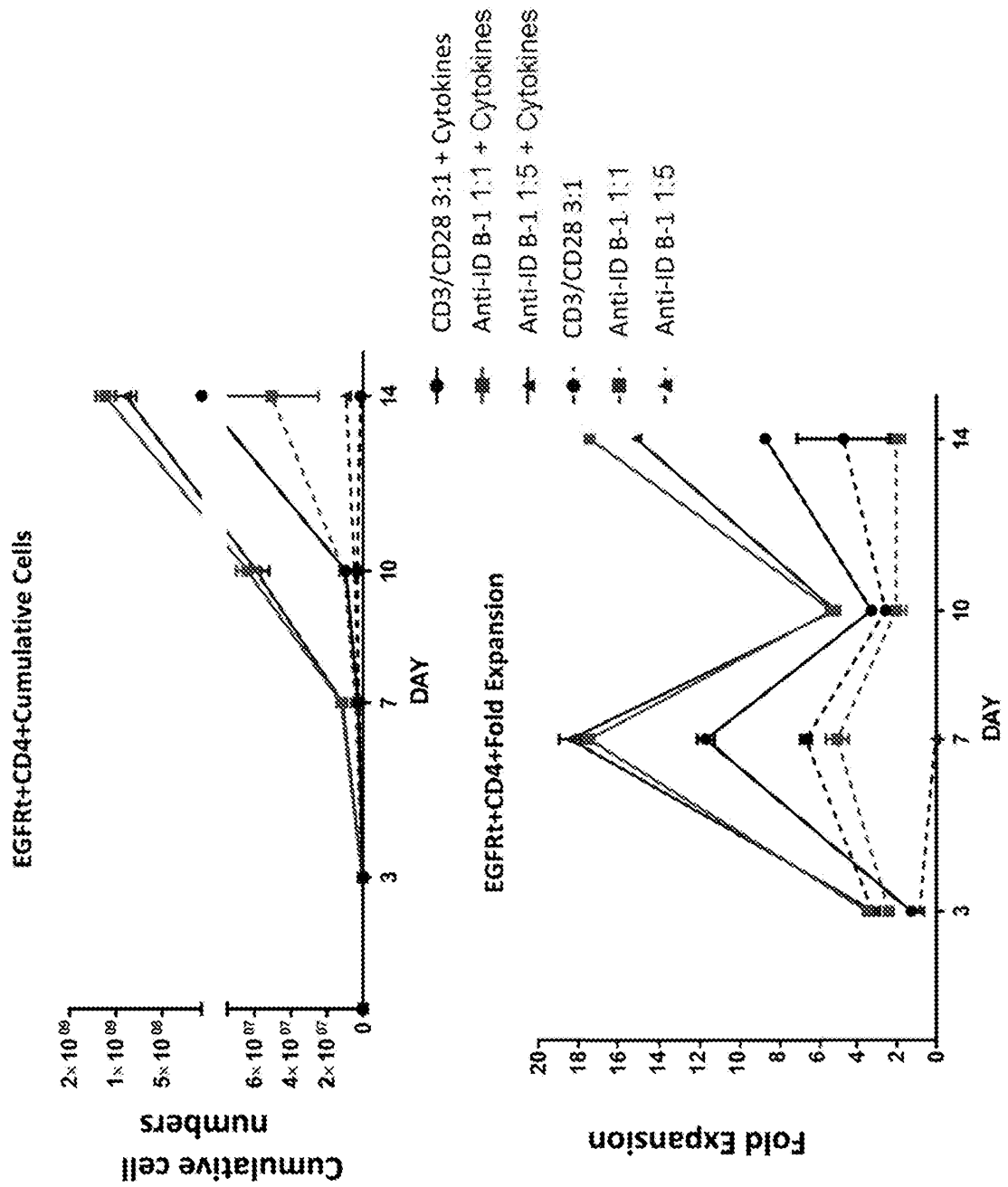
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FIG. 5



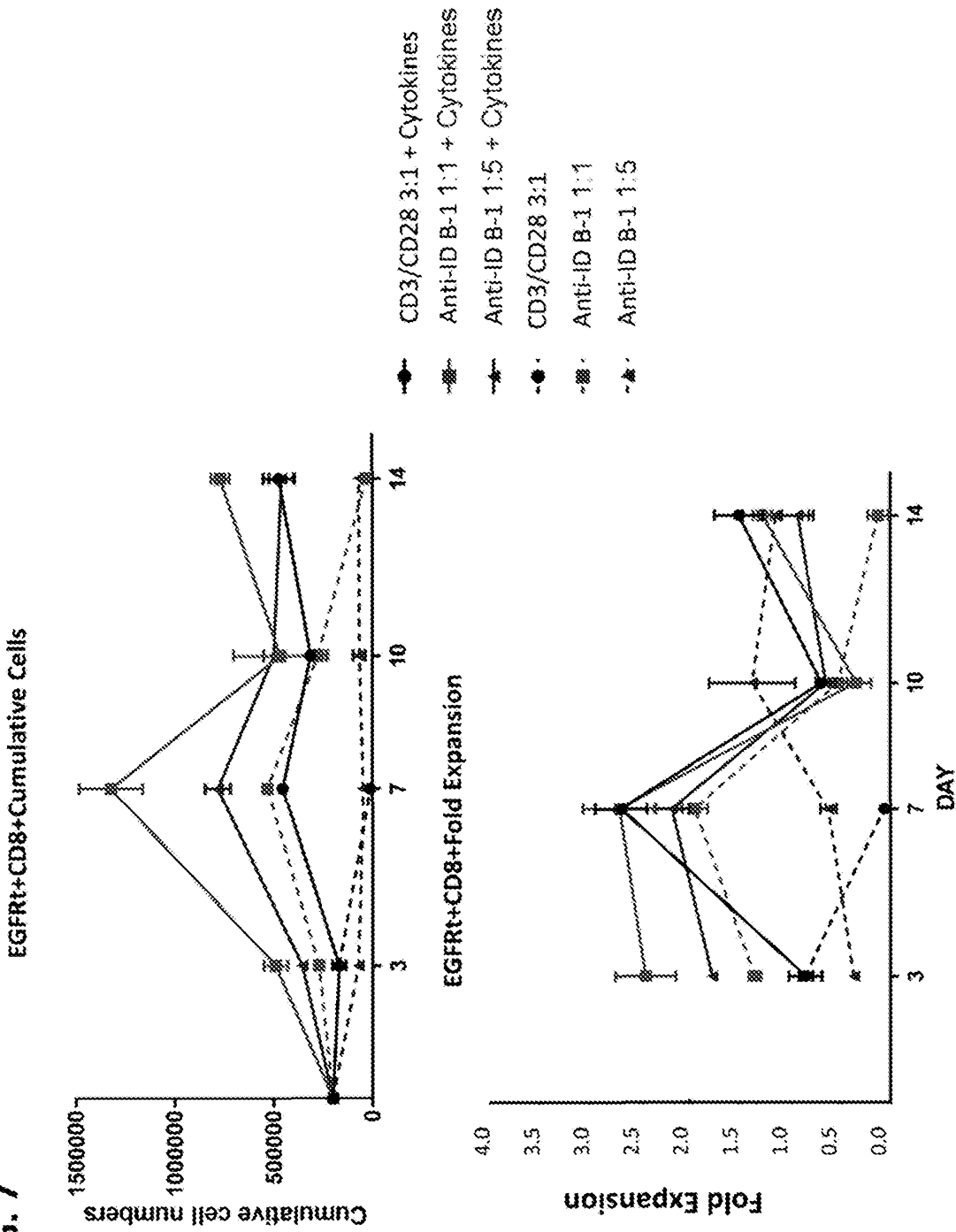
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FIG. 6

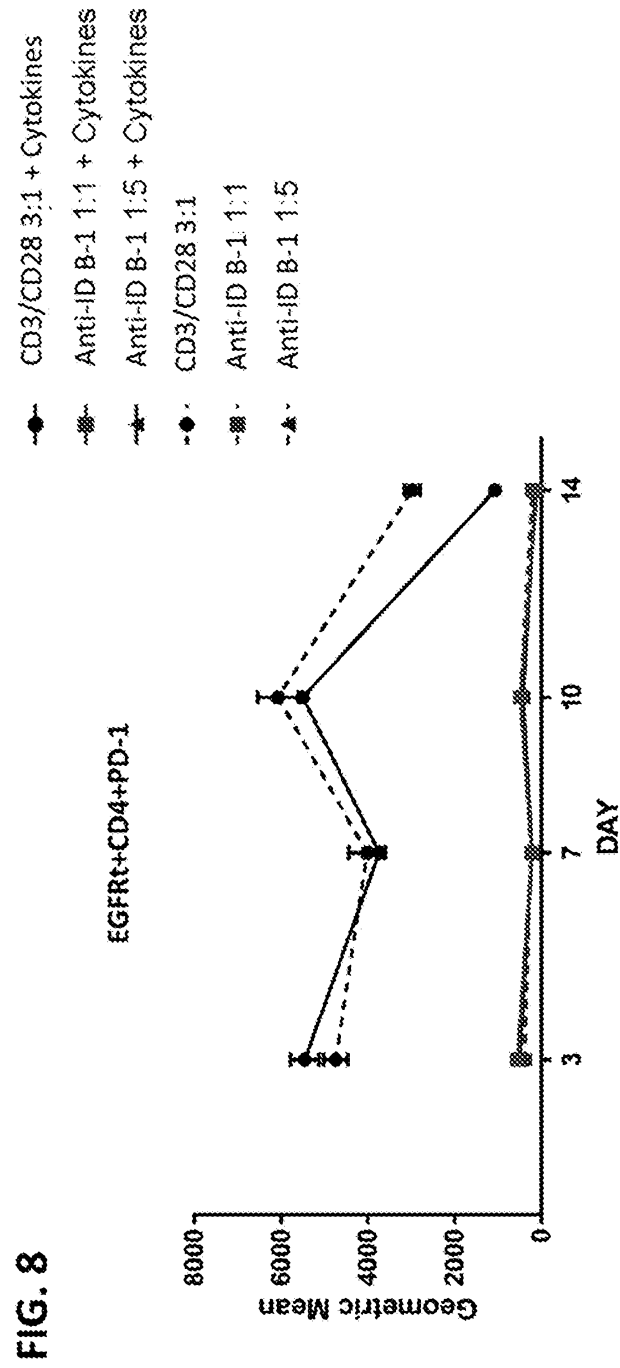


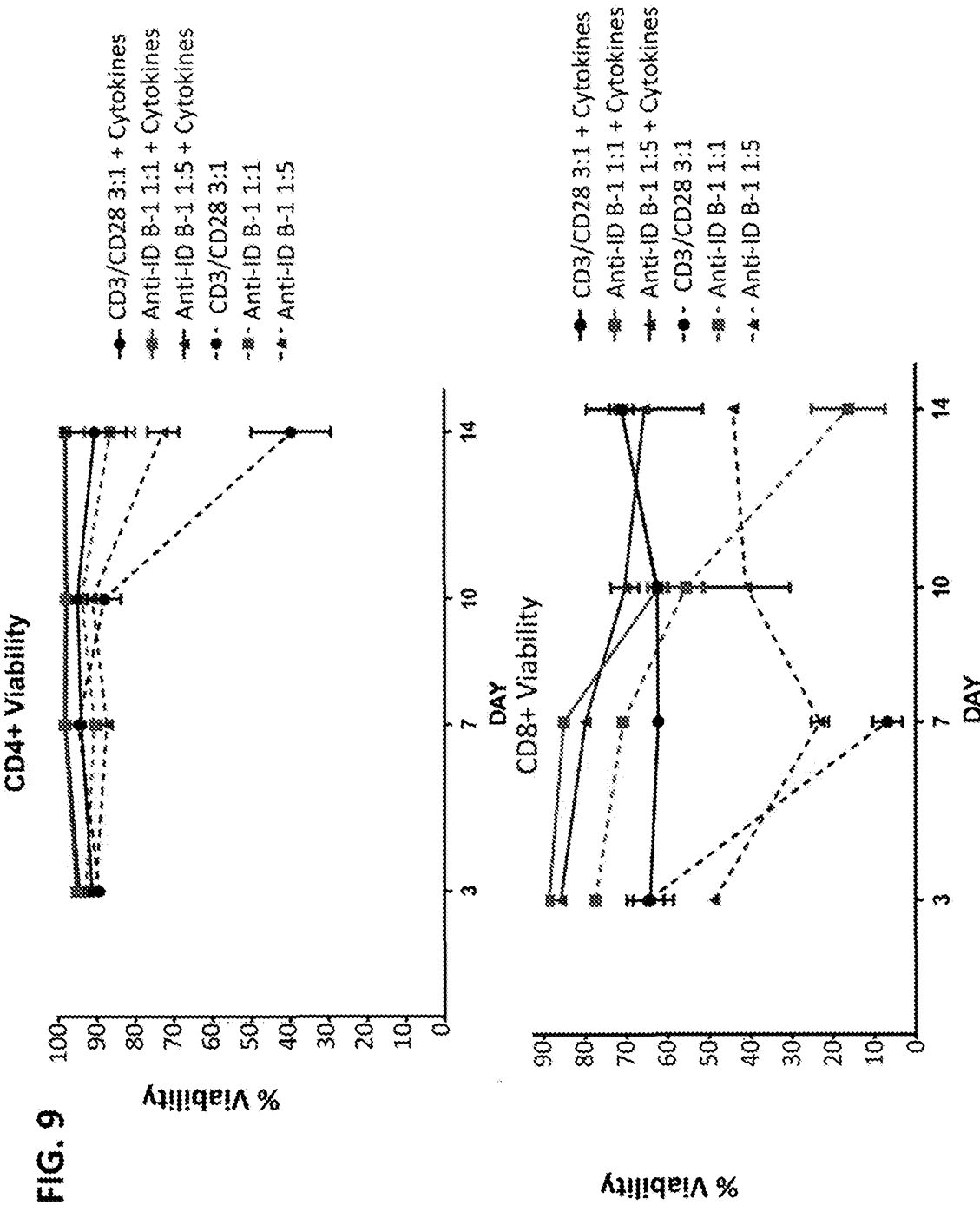
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FIG. 7

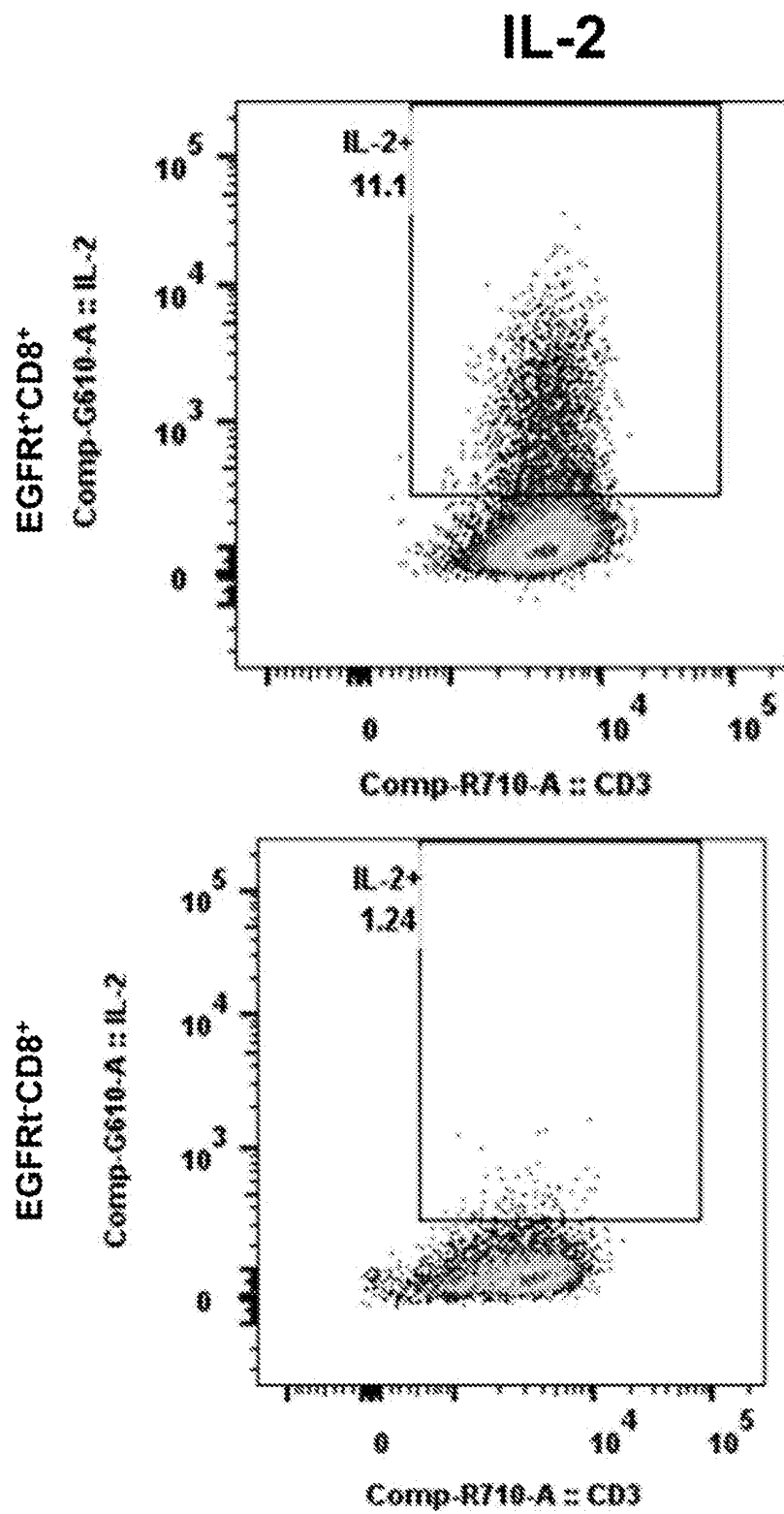


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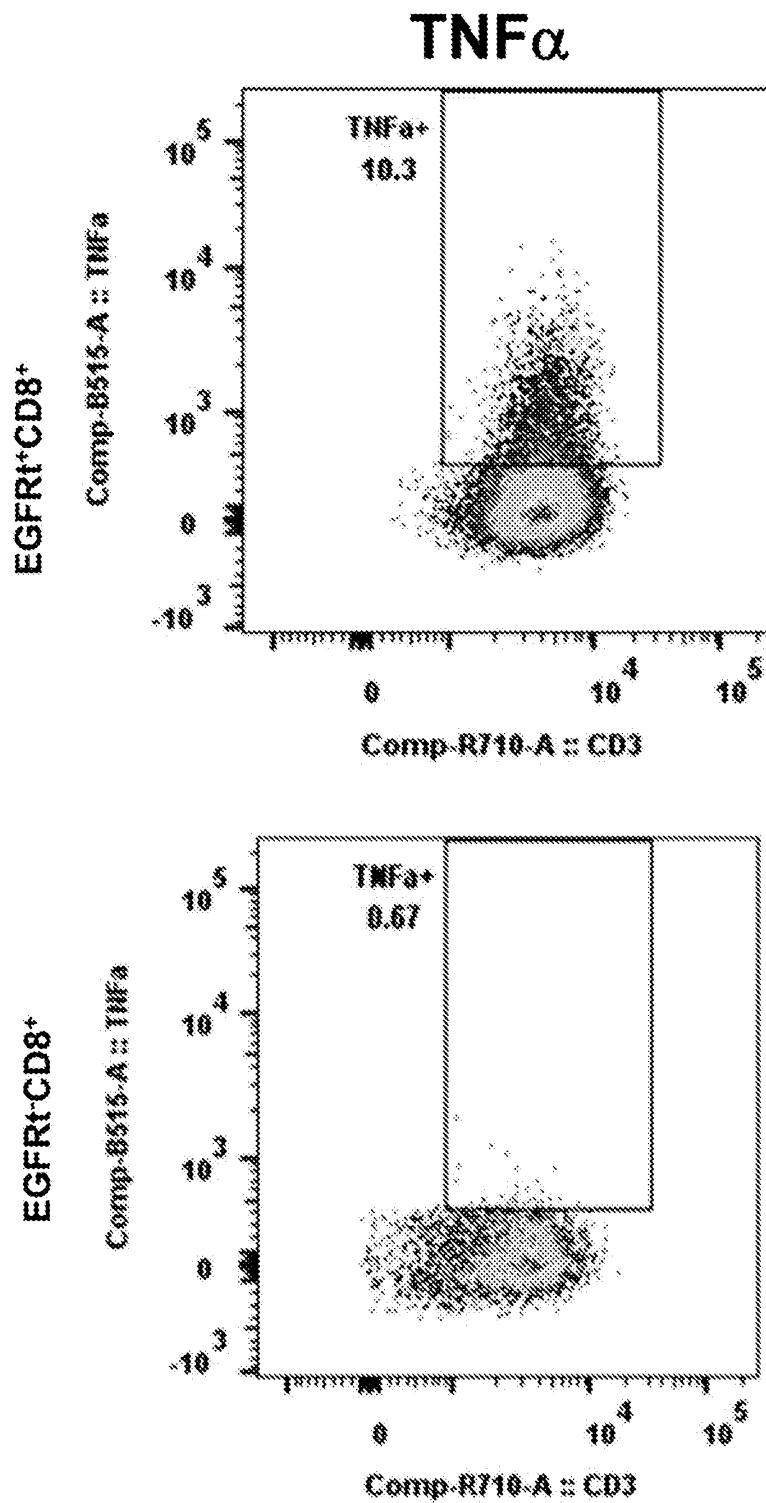




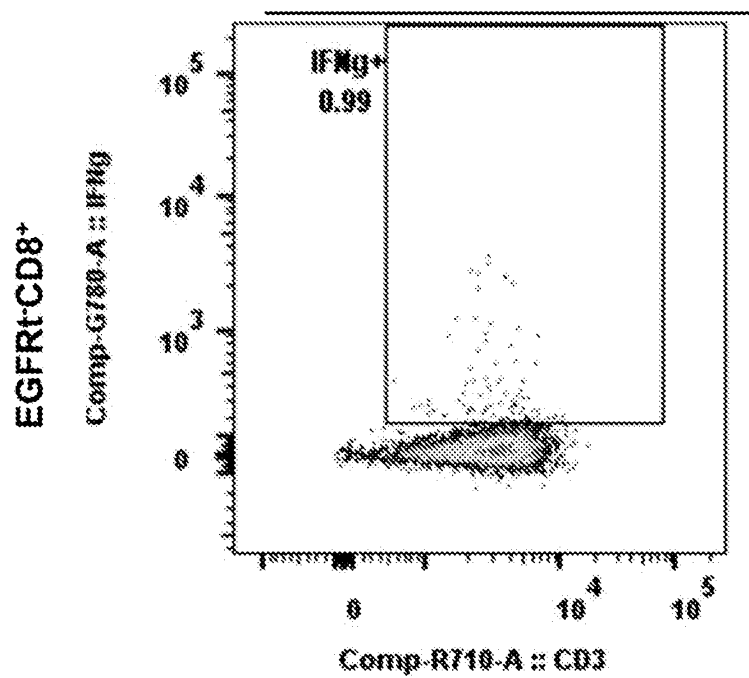
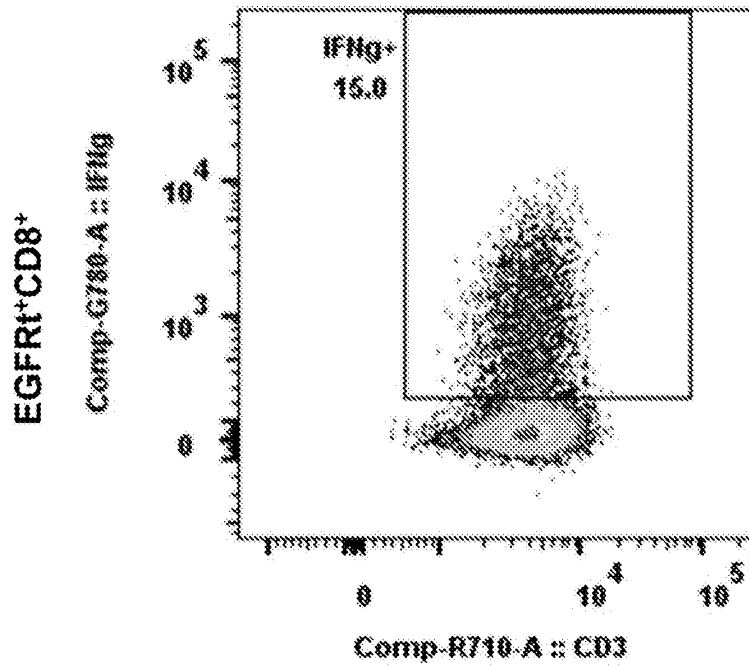
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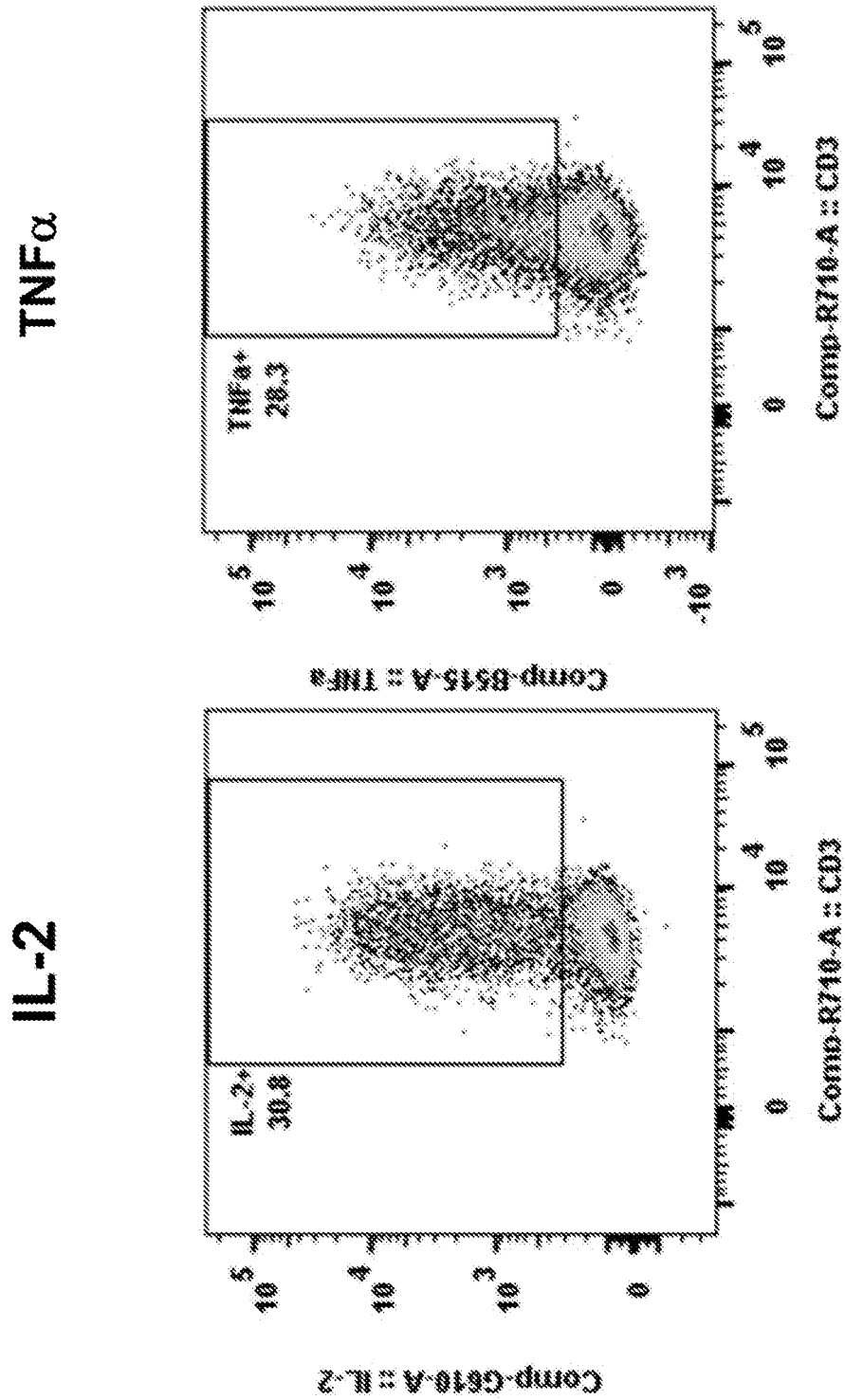


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**FIG. 10A Cont.**

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IFN γ **FIG. 10A Cont.**



K562-CD19 (E:T = 1:2)

FIG. 10B

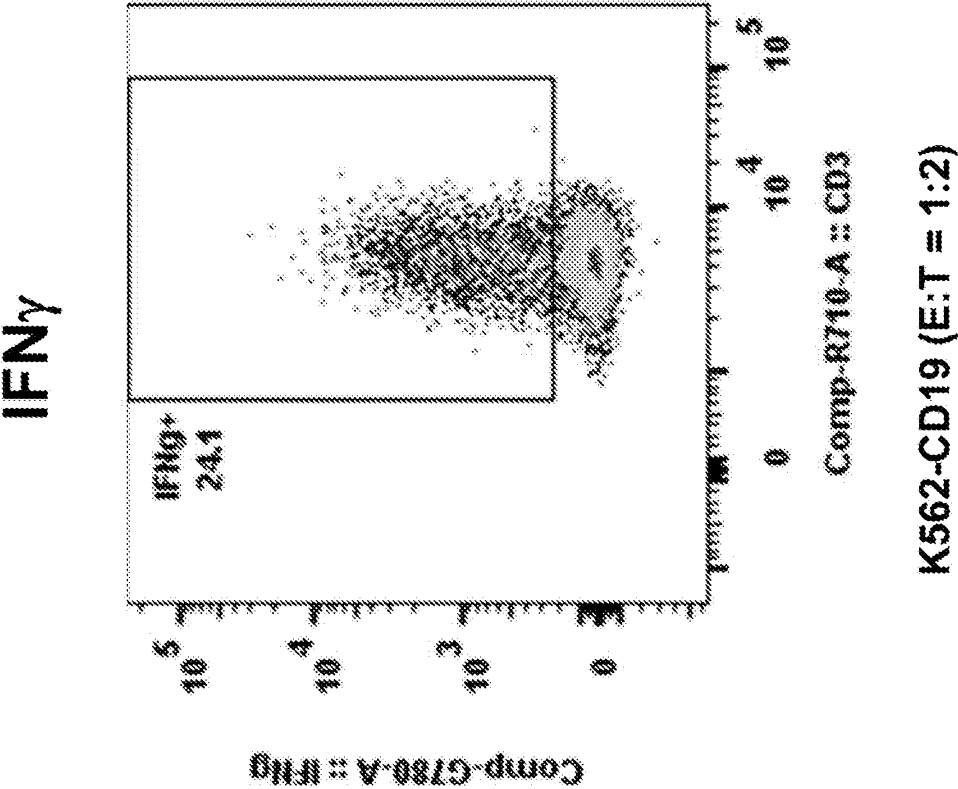


FIG. 10B Cont.

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FIG. 11

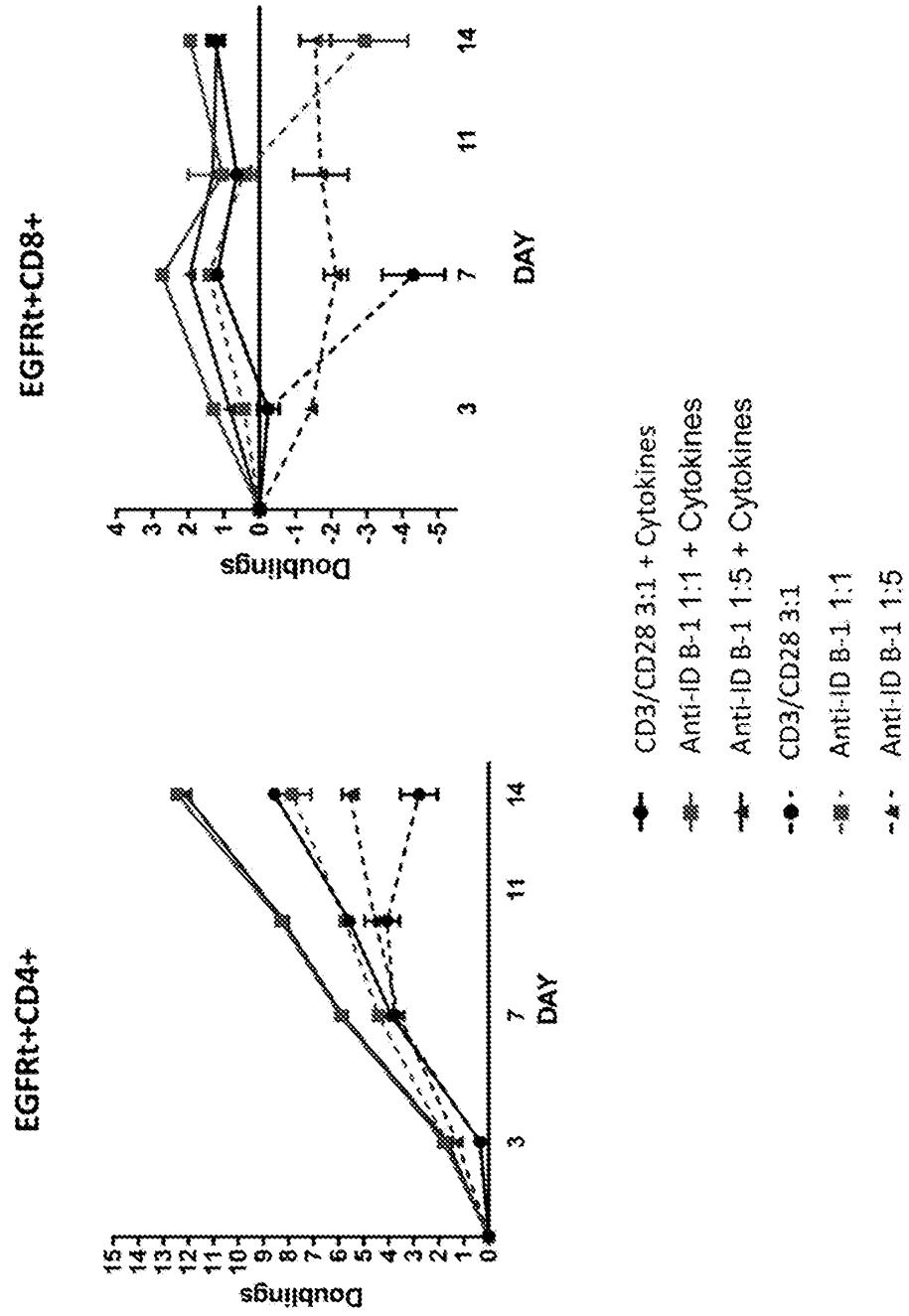
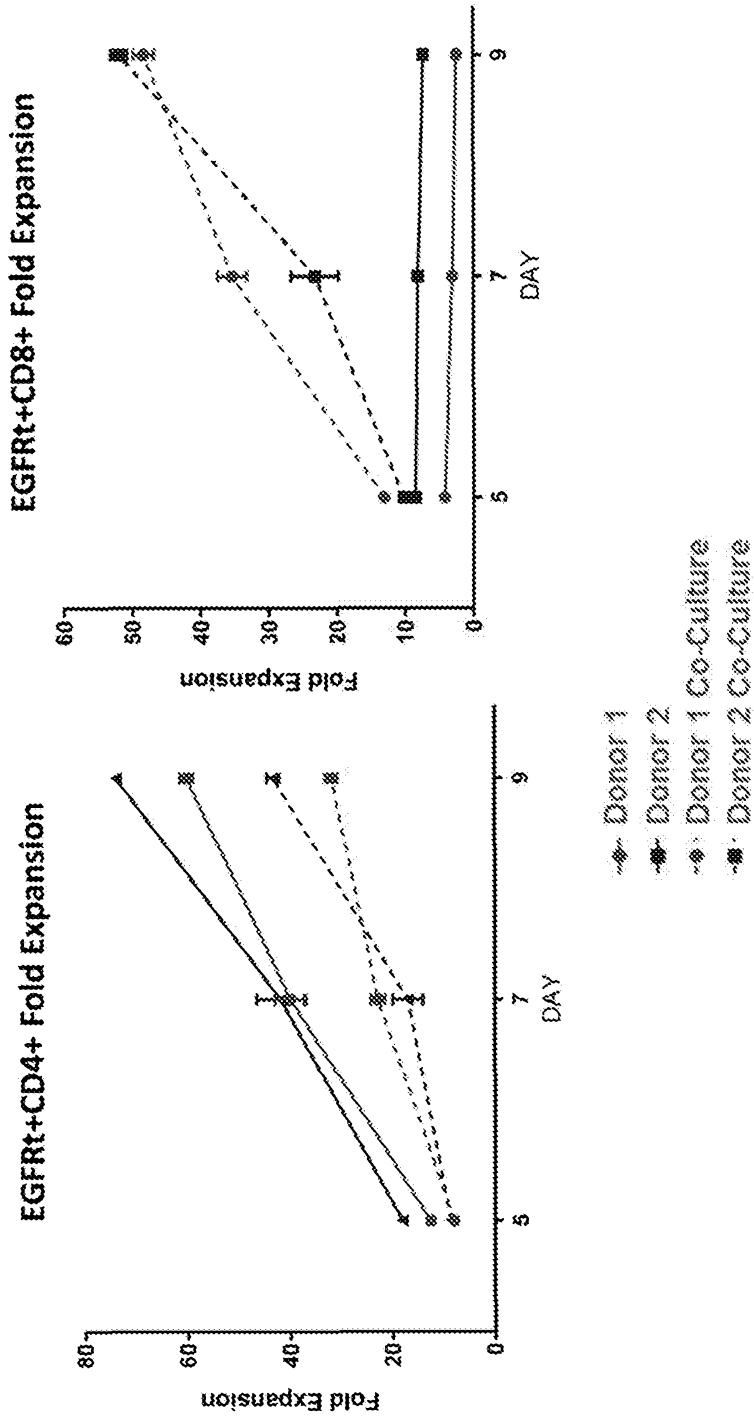


FIG. 12A



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FIG. 12B

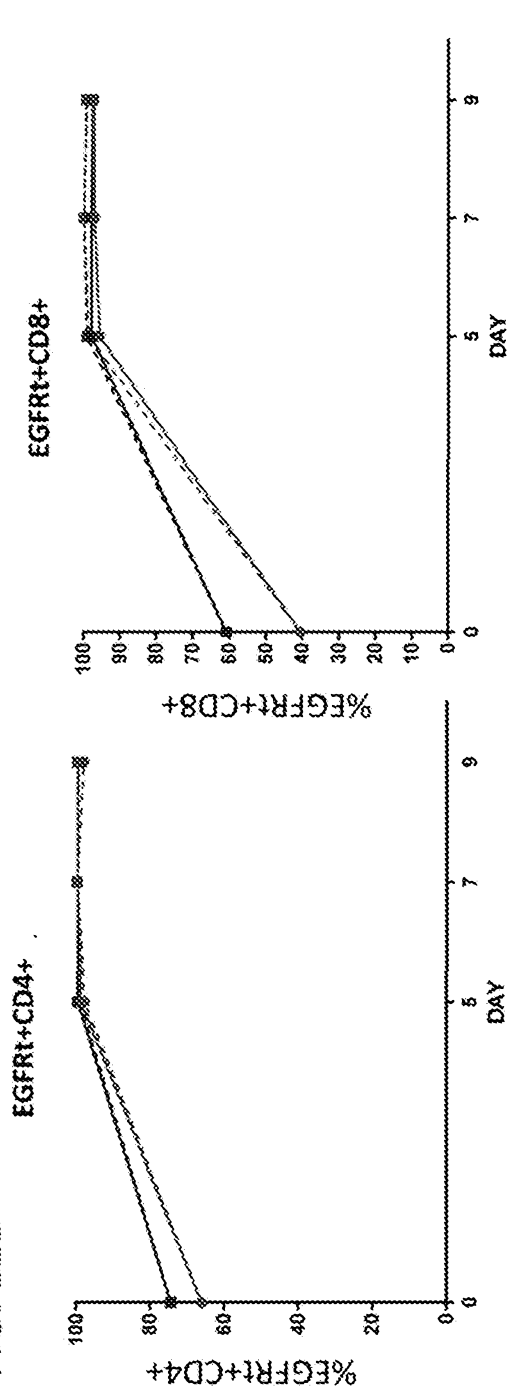


FIG. 12C

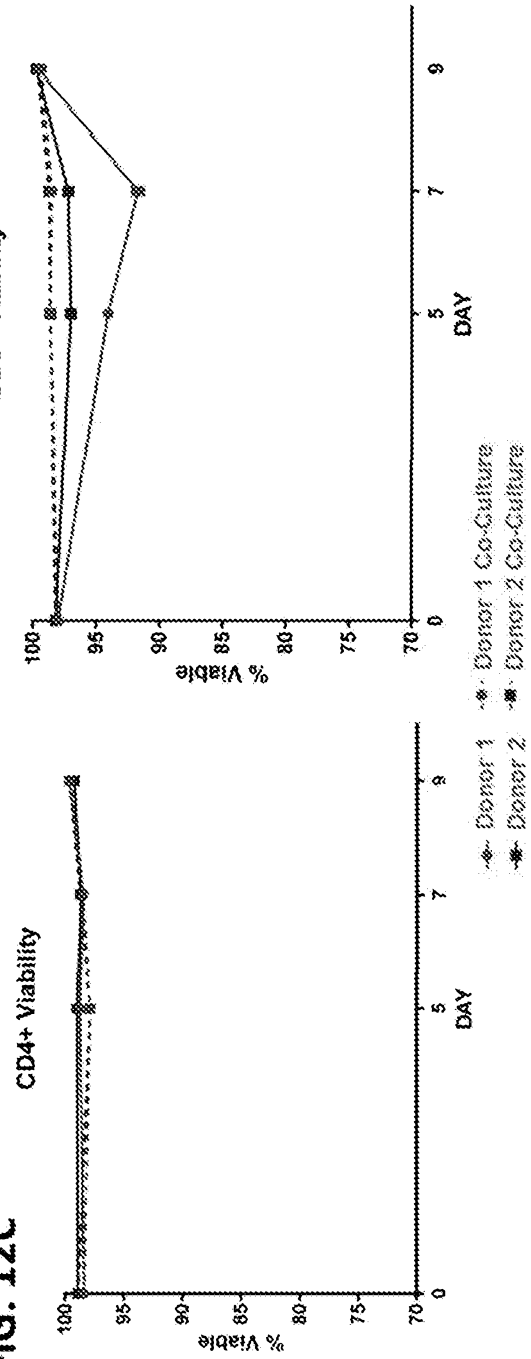


FIG. 13A

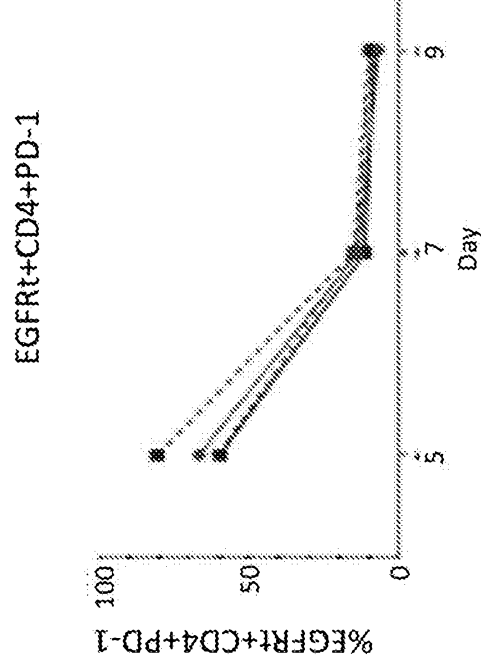
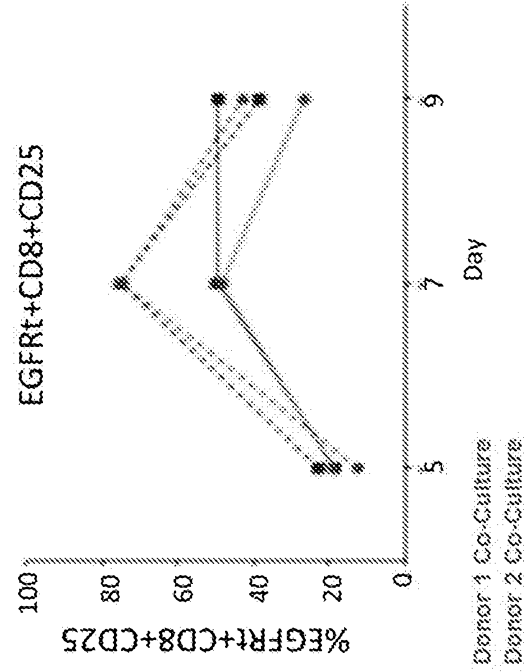
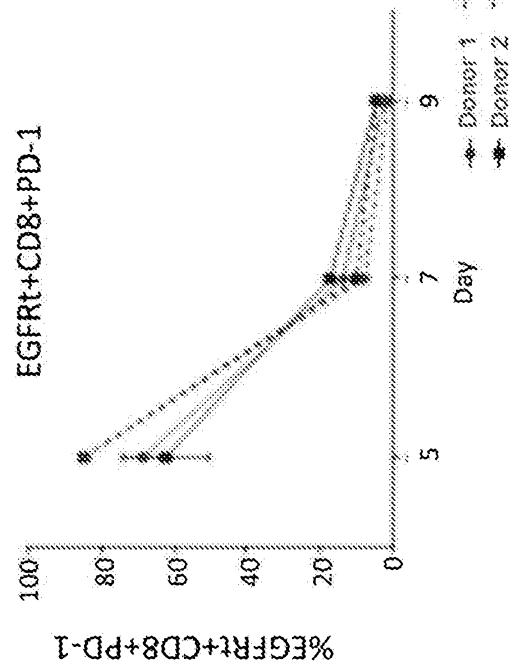
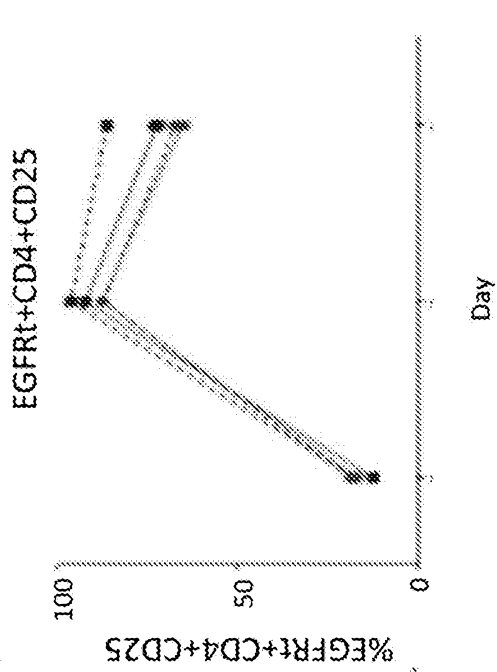
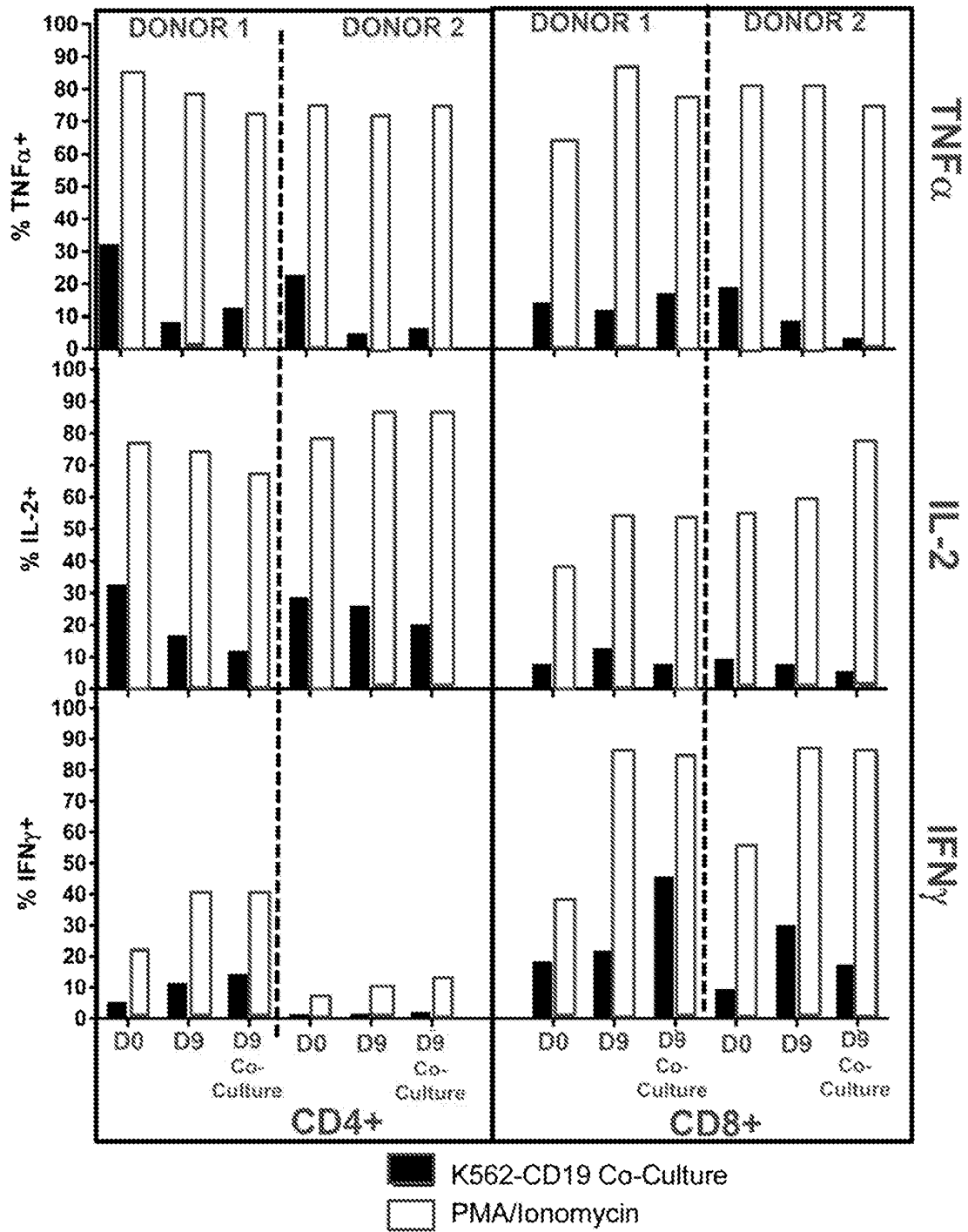


FIG. 13B

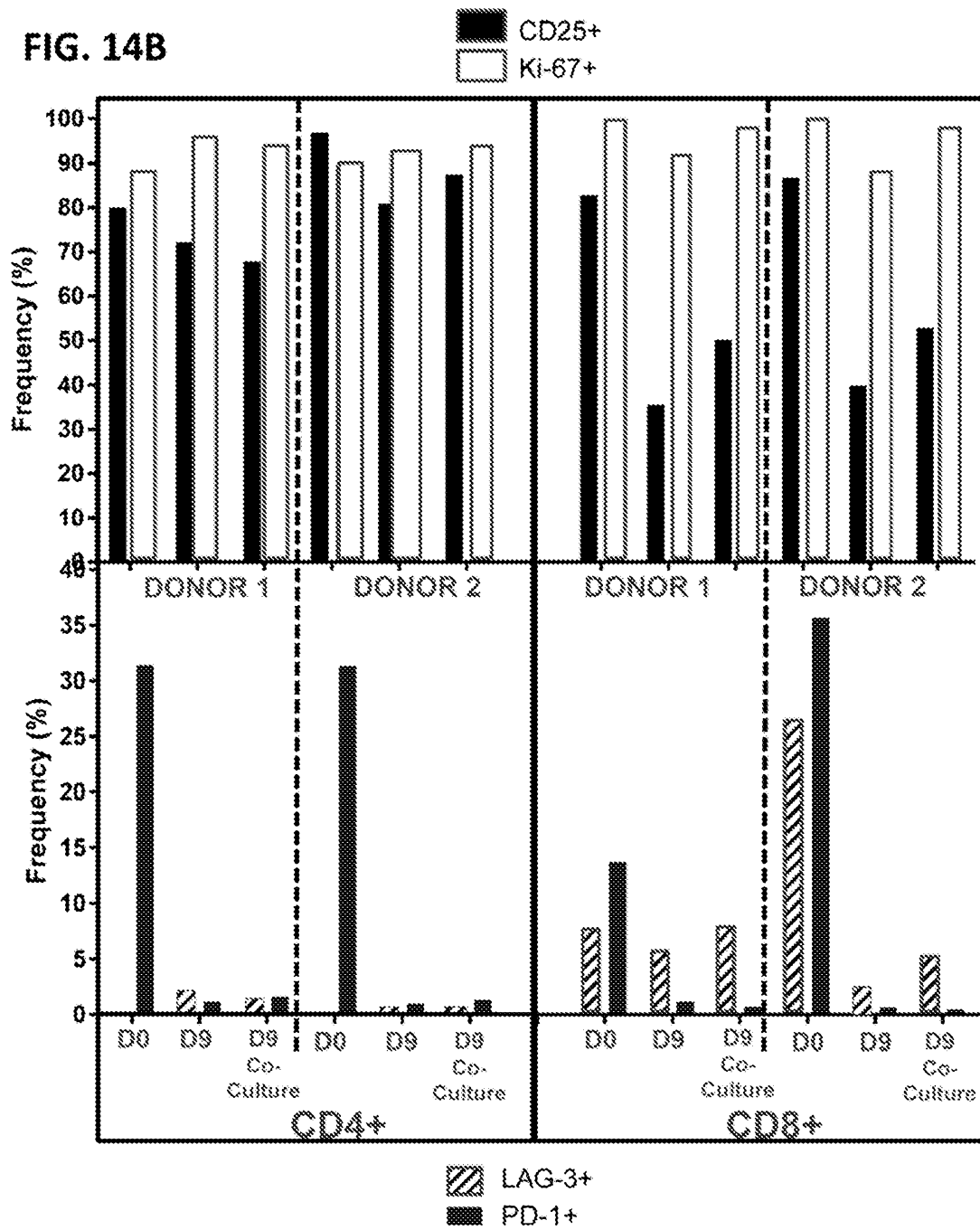


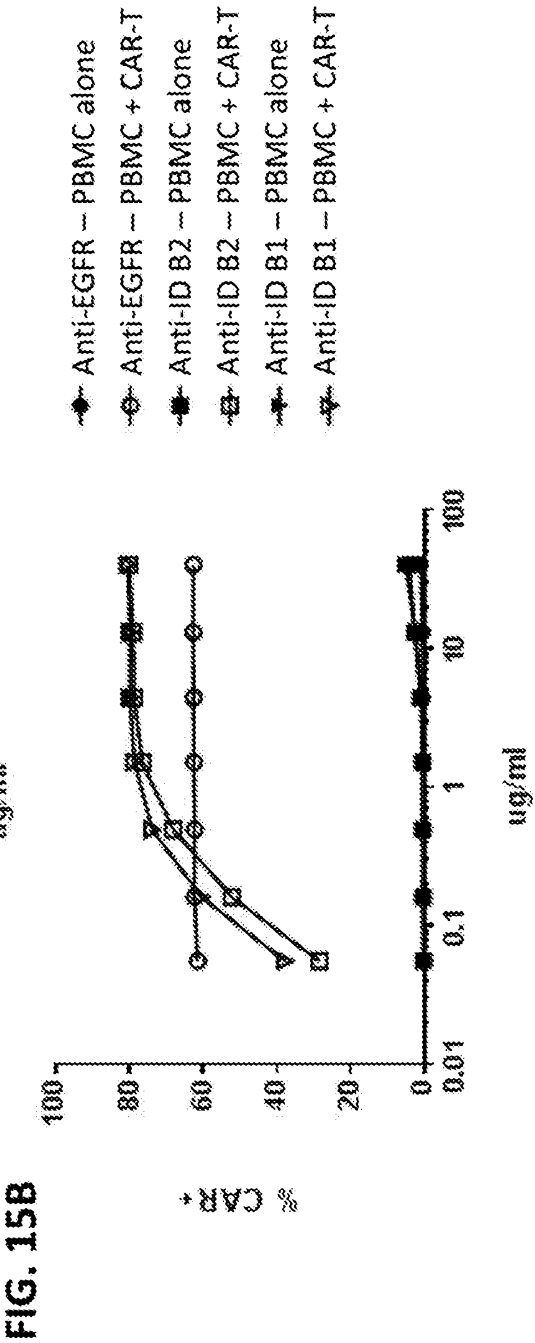
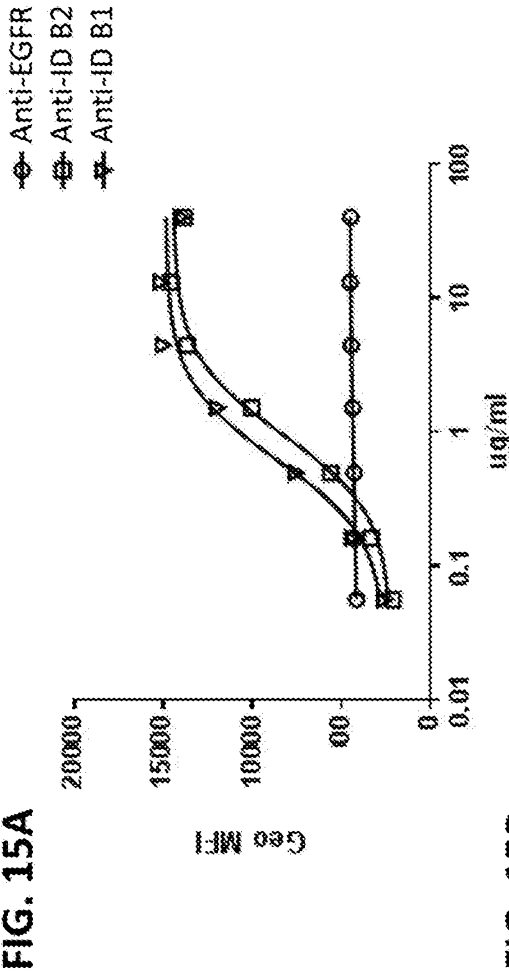
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FIG. 14A



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SEQUENCE LISTING

<110> Juno Therapeutics, Inc.
 HAUSKINS, Colin
 HEIPEL, Mark D.
 SUTHERLAND, Claire L.
 SATHALIYA, Taher
 SMITH, Jeff

<120> ANTI-IDIOTYPIC ANTIBODIES AND RELATED
 METHODS

<130> 735042006540

<140> Not Yet Assigned

<141> Concurrently Herewith

<150> 62/369,008

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<160> 164

<170> FastSEQ for windows Version 4.0

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<211> 122

<212> PRT

<213> Artificial Sequence

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Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Asp	Tyr	Thr	Phe	Thr	Ser	Tyr
		20						25				30			
Trp	Met	His	Trp	Val	Arg	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			
Gly	Asn	Ile	Tyr	Pro	Gly	Ser	Gly	Gly	Thr	Asn	Tyr	Asp	Glu	Lys	Phe
	50					55				60					
Lys	Arg	Lys	Ala	Thr	Leu	Thr	Val	Asp	Thr	Ser	Ser	Ser	Thr	Ala	Tyr
	65				70					75					80
Met	Gln	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Thr	Arg	Glu	Val	Thr	Thr	Val	Ala	Tyr	Tyr	Tyr	Ser	Met	Asp	Tyr	Trp
			100					105					110		
Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser						
		115					120								

<210> 2

<211> 324

<212> PRT

<213> Artificial Sequence

<220>

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<400> 2

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1				5					10					15	
Ala	Gln	Thr	Asn	Ser	Met	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	Gly	Tyr
		20						25					30		
Phe	Pro	Glu	Pro	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Ser	Leu	Ser	Ser
		35					40					45			
Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Asp	Leu	Tyr	Thr	Leu

735042006540SeqList.txt

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65      70      75      80
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
85      90      95
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
100      105      110
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
115      120      125
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
130      135      140
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
145      150      155      160
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
165      170      175
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
180      185      190
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
195      200      205
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
210      215      220
Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
225      230      235      240
Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
245      250      255
Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
260      265      270
Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
275      280      285
Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
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Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
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Ser Pro Gly Lys

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 <213> Artificial Sequence

<220>
 <223> anti-ID heavy chain

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20      25      30
Trp Met His Trp Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35      40      45
Gly Asn Ile Tyr Pro Gly Ser Gly Gly Thr Asn Tyr Asp Glu Lys Phe
50      55      60
Lys Arg Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65      70      75      80
Met Gln Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85      90      95
Thr Arg Glu Val Thr Thr Val Ala Tyr Tyr Tyr Ser Met Asp Tyr Trp
100      105      110
Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro
115      120      125
Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met
130      135      140
Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr
145      150      155      160
Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
165      170      175

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735042006540seqList.txt

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Pro	Ser	Ser	Thr	Trp	Pro	Ser	Glu	Thr	Val	Thr	Cys	Asn	Val	Ala	His
		195					200					205			
Pro	Ala	Ser	Ser	Thr	Lys	Val	Asp	Lys	Lys	Ile	Val	Pro	Arg	Asp	Cys
	210					215					220				
Gly	Cys	Lys	Pro	Cys	Ile	Cys	Thr	Val	Pro	Glu	Val	Ser	Ser	Val	Phe
	225				230					235					240
Ile	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Val	Leu	Thr	Ile	Thr	Leu	Thr	Pro
				245					250					255	
Lys	Val	Thr	Cys	Val	Val	Val	Asp	Ile	Ser	Lys	Asp	Asp	Pro	Glu	Val
			260					265					270		
Gln	Phe	Ser	Trp	Phe	Val	Asp	Asp	Val	Glu	Val	His	Thr	Ala	Gln	Thr
		275					280					285			
Gln	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Phe	Arg	Ser	Val	Ser	Glu
	290					295					300				
Leu	Pro	Ile	Met	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Phe	Lys	Cys
	305				310					315					320
Arg	Val	Asn	Ser	Ala	Ala	Phe	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser
				325					330					335	
Lys	Thr	Lys	Gly	Arg	Pro	Lys	Ala	Pro	Gln	Val	Tyr	Thr	Ile	Pro	Pro
			340					345					350		
Pro	Lys	Glu	Gln	Met	Ala	Lys	Asp	Lys	Val	Ser	Leu	Thr	Cys	Met	Ile
		355					360					365			
Thr	Asp	Phe	Phe	Pro	Glu	Asp	Ile	Thr	Val	Glu	Trp	Gln	Trp	Asn	Gly
	370					375					380				
Gln	Pro	Ala	Glu	Asn	Tyr	Lys	Asn	Thr	Gln	Pro	Ile	Met	Asp	Thr	Asp
	385				390					395					400
Gly	Ser	Tyr	Phe	Val	Tyr	Ser	Lys	Leu	Asn	Val	Gln	Lys	Ser	Asn	Trp
			405						410					415	
Glu	Ala	Gly	Asn	Thr	Phe	Thr	Cys	Ser	Val	Leu	His	Glu	Gly	Leu	His
			420					425					430		
Asn	His	His	Thr	Glu	Lys	Ser	Leu	Ser	His	Ser	Pro	Gly	Lys		
		435					440					445			

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 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID HC signal sequence

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 1 5 10 15
 Val His Ser

<210> 5
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID VL

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 1 5 10 15
 Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45
 Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

735042006540SeqList.txt

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Lys Thr Val Pro Phe
85 90 95
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 6
<211> 107
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<213> Artificial Sequence

<220>
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1 5 10 15
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
20 25 30
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
35 40 45
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
50 55 60
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
65 70 75 80
Arg His Asn Asn Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
85 90 95
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
100 105

<210> 7
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID light chain

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20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45
Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Lys Thr Val Pro Phe
85 90 95
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala
100 105 110
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
130 135 140
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145 150 155 160
Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
165 170 175
Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Asn Tyr
180 185 190
Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
195 200 205

Phe Asn Arg Asn Glu Cys
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<210> 8
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID LC signal sequence

<400> 8
Met Met Ser Ser Ala Gln Phe Leu Gly Leu Leu Leu Leu Cys Phe Gln
1 5 10 15
Gly Thr Arg Cys
20

<210> 9
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID HC-CDR1

<400> 9
Ser Tyr Trp Met His
1 5

<210> 10
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID HC-CDR2

<400> 10
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1 5 10 15
Arg

<210> 11
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID HC-CDR3

<400> 11
Glu Val Thr Thr Val Ala Tyr Tyr Tyr Ser Met Asp Tyr
1 5 10

<210> 12
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID LC-CDR1

<400> 12
 Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn
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 <211> 7
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 <213> Artificial Sequence

<220>
 <223> anti-ID LC-CDR2

<400> 13
 Tyr Thr Ser Arg Leu His Ser
 1 5

<210> 14
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID LC-CDR3

<400> 14
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<210> 15
 <211> 366
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID VH

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 cctggacaag gccttgagtg gattggaaat atttatcctg gtagtggtgg tactaactac 180
 gatgagaagt tcaagaggaa ggccacactg actgtagaca catcctccag cacagcctac 240
 atgcagctcc gcagcctgac atctgaggac tctgcggtct attactgtac aagagaggtt 300
 actacagtag cttattacta ttctatggac tactggggtc aaggaacctc agtcaccgctc 366
 tcctca

<210> 16
 <211> 975
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID CH

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 tccatggtga ccctgggatg cctggtcaag ggctatttcc ctgagccagt gacagtgacc 120
 tggaactctg gatccctgtc cagcgggtgtg cacaccttcc cagctgtcct gcagtctgac 180
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 acctgcaacg ttgcccaccc ggccagcagc accaagggtg acaagaaaat tgtgcccagg 300
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 gtgcacacag ctgagacgca accccgggag gtagcattca acagcacttt ccgctcagtc 540
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 aacagtgcag ctttcctgc cccatcgag aaaaccatct ccaaaaccaa aggagaccg 660

735042006540seqList.txt

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aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct 840
tacttcgtct acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc 900
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tctcctggta aatga 975

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<210> 17
 <211> 1341
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID heavy chain

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cctggacaag gccttgagtg gattggaaat atttatcctg gtagtggtgg tactaactac 180
gatgagaagt tcaagaggaa ggccacactg actgtagaca catcctccag cacagcctac 240
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tcctcagcca aaacgacacc cccatctgtc tatccactgg cccctggatc tgctgcccaa 420
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<210> 18
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID HC signal sequence

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 <211> 321
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 <213> Artificial Sequence

<220>
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gatggaactg ttaaactcct gatctactac acatcaagat tacactcagg agtcccatca 180
aggttcagtg gcagtgggtc tggaaacagat tattctctca ccattagcaa cctggagcaa 240
gaagatattg ccacttactt ttgtcagcag ggtaaaacgg ttccattcac gttcggctcg 300
gggacaaagt tggaaataaa a 321

```

<210> 20

<211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID CL

<400> 20
 cgggctgatg ctgcaccaac tgtatccatc ttcccaccat ccagtgagca gttaacatct 60
 ggaggtgcct cagtcgtgtg cttcttgaac aacttctacc ccaaagacat caatgtcaag 120
 tggaagattg atggcagtga acgacaaaat ggcgtcctga acagttggac tgatcaggac 180
 agcaaagaca gcacctacag catgagcagc accctcacgt tgaccaagga cgagtatgaa 240
 cgacataaca actatacctg tgaggccact cacaagacat caacttcacc cattgtcaag 300
 agcttcaaca ggaatgagtg ttag 324

<210> 21
 <211> 645
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID light chain

<400> 21
 gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga cagagtcacc 60
 atcagttgca gggcaagtca ggacattagc aattatttaa actgggatca gcagaaacca 120
 gatggaactg ttaaaactcct gatctactac acatcaagat tacactcagg agtcccatca 180
 aggttcagtg gcagtggtgc tggaacagat tattctctca ccattagcaa cctggagcaa 240
 gaagatattg ccacttactt ttgtcagcag ggtaaaacgg ttccattcac gttcggctcg 300
 gggacaaagt tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca 360
 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac 420
 cccaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg 480
 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg 540
 ttgaccaagg acgagtatga acgacataac aactatacct gtgaggccac tcacaagaca 600
 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag 645

<210> 22
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID LC signal sequence

<400> 22
 atgatgtcct ctgctcagtt ccttggtctc ctgttgctct gttttcaagg taccagatgt 60

<210> 23
 <211> 122
 <212> PRT
 <213> Mus musculus

<220>
 <223> SJ25C1 VH

<400> 23
 Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60
 Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys

735042006540SeqList.txt

Ala Arg Lys Thr 85 Ile Ser Ser Val Val 90 Asp Phe Tyr Phe Asp 95 Tyr Trp
 100 105 110
 Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 24
 <211> 108
 <212> PRT
 <213> Mus musculus

<220>
 <223> SJ25C1 VL

<400> 24
 Asp Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly
 1 5 10 15
 Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
 20 25 30
 Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile
 35 40 45
 Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
 65 70 75 80
 Lys Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr
 85 90 95
 Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

<210> 25
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Linker

<400> 25
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15

<210> 26
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4GS linker

<400> 26
 Gly Gly Gly Gly Ser
 1 5

<210> 27
 <211> 39
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> An extracellular portion of CD28

<400> 27
 Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn

735042006540SeqList.txt

1 5 10 15
 Gly Thr Ile Ile Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu
 20 25 30
 Phe Pro Gly Pro Ser Lys Pro
 35

<210> 28
 <211> 245
 <212> PRT
 <213> Mus musculus

<220>
 <223> SJ25C1 scFv

<400> 28
 Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60
 Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95
 Ala Arg Lys Thr Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Tyr Trp
 100 105 110
 Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly
 115 120 125
 Gly Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser
 130 135 140
 Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val Thr Cys
 145 150 155 160
 Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln Gln Lys
 165 170 175
 Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr Arg Asn
 180 185 190
 Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
 195 200 205
 Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp Tyr Phe
 210 215 220
 Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly Thr Lys
 225 230 235 240
 Leu Glu Ile Lys Arg
 245

<210> 29
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 3GS linker

<400> 29
 Gly Gly Gly Ser
 1

<210> 30
 <211> 120
 <212> PRT
 <213> Mus musculus

<220>

<223> FMC63 VH

<400> 30

```

Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
 1          5          10          15
Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr
          20          25          30
Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu
          35          40          45
Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys
          50          55          60
Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65          70          75          80
Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
          85          90          95
Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln
          100          105          110
Gly Thr Ser Val Thr Val Ser Ser
          115          120

```

<210> 31

<211> 107

<212> PRT

<213> Mus musculus

<220>

<223> FMC63 VL

<400> 31

```

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1          5          10          15
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
          20          25          30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
          35          40          45
Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
          50          55          60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65          70          75          80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
          85          90          95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
          100          105

```

<210> 32

<211> 220

<212> PRT

<213> Artificial Sequence

<220>

<223> IgG1 Fc lacking hinge

<400> 32

```

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 1          5          10          15
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
          20          25          30
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
          35          40          45
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
          50          55          60
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
65          70          75          80
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
          85          90          95

```

735042006540SeqList.txt

```

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
100 105 110
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
115 120 125
Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
130 135 140
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
145 150 155 160
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
165 170 175
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
180 185 190
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
195 200 205
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215 220

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<210> 33
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Linker

<400> 33
 Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr
 1 5 10 15
 Lys Gly

<210> 34
 <211> 245
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> FMC63 scFv

<400> 34
 Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15
 Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45
 Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Ser Thr Gly
 100 105 110
 Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly Glu Val Lys
 115 120 125
 Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser
 130 135 140
 Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly Val Ser
 145 150 155 160
 Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile
 165 170 175
 Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser Arg Leu
 180 185 190
 Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn
 195 200 205

735042006540SeqList.txt

Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys His Tyr
 210 220
 Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
 225 230 235 240
 Val Thr Val Ser Ser
 245

<210> 35
 <211> 465
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> FMC63 reagent

<400> 35
 Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15
 Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45
 Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Ser Thr Ser Gly
 100 105 110
 Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly Glu Val Lys
 115 120 125
 Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser
 130 135 140
 Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly Val Ser
 145 150 155 160
 Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile
 165 170 175
 Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser Arg Leu
 180 185 190
 Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn
 195 200 205
 Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys His Tyr
 210 215 220
 Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
 225 230 235 240
 Val Thr Val Ser Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
 245 250 255
 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 260 265 270
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 275 280 285
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 290 295 300
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 305 310 315 320
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 325 330 335
 Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 340 345 350
 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 355 360 365
 Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
 370 375 380
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 385 390 395 400
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu

735042006540SeqList.txt

405 410 415
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 420 425 430
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 435 440 445
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 450 455 460
 Lys
 465

<210> 36
 <211> 122
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-1 VH

<400> 36
 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30
 Tyr Met Lys Trp Val Lys Gln Cys His Gly Lys Ser Leu Glu Trp Ile
 35 40 45
 Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Asp Tyr Asn Gln Asn Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Gly Asn Asn Tyr Gly Ser Arg Asp Ala Met Asp Tyr Trp
 100 105 110
 Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> 37
 <211> 330
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-1 CH

<400> 37
 Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
 1 5 10 15
 Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
 20 25 30
 Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
 35 40 45
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
 50 55 60
 Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
 65 70 75 80
 Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
 85 90 95
 Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
 100 105 110
 Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
 115 120 125
 Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
 130 135 140
 Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
 145 150 155 160
 Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg

735042006540SeqList.txt

				165					170					175	
Glu	Asp	Tyr	Asn	Ser	Thr	Leu	Arg	Val	Val	Ser	Ala	Leu	Pro	Ile	Gln
			180					185					190		
His	Gln	Asp	Trp	Met	Ser	Gly	Lys	Glu	Phe	Lys	Cys	Lys	Val	Asn	Asn
		195					200					205			
Lys	Asp	Leu	Pro	Ala	Pro	Ile	Glu	Arg	Thr	Ile	Ser	Lys	Pro	Lys	Gly
		210				215					220				
Ser	Val	Arg	Ala	Pro	Gln	Val	Tyr	Val	Leu	Pro	Pro	Pro	Glu	Glu	Glu
225					230					235					240
Met	Thr	Lys	Lys	Gln	Val	Thr	Leu	Thr	Cys	Met	Val	Thr	Asp	Phe	Met
			245						250					255	
Pro	Glu	Asp	Ile	Tyr	Val	Glu	Trp	Thr	Asn	Asn	Gly	Lys	Thr	Glu	Leu
			260					265					270		
Asn	Tyr	Lys	Asn	Thr	Glu	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Tyr	Phe
		275					280					285			
Met	Tyr	Ser	Lys	Leu	Arg	Val	Glu	Lys	Lys	Asn	Trp	Val	Glu	Arg	Asn
		290				295					300				
Ser	Tyr	Ser	Cys	Ser	Val	Val	His	Glu	Gly	Leu	His	Asn	His	His	Thr
305					310					315					320
Thr	Lys	Ser	Phe	Ser	Arg	Thr	Pro	Gly	Lys						
				325					330						

```
<210> 38
<211> 452
<212> PRT
<213> Artificial Sequence
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<220>
<223> anti-ID B-1 heavy chain

<400>	38															
Glu 1	Val	Gln	Leu	Gln 5	Gln	Ser	Gly	Pro	Glu 10	Leu	Val	Lys	Pro	Gly 15	Ala	
Ser	Val	Lys	Met 20	Ser	Cys	Lys	Ala	Ser 25	Gly	Tyr	Thr	Phe	Thr 30	Asp	Tyr	
Tyr	Met	Lys 35	Trp	Val	Lys	Gln	Cys 40	His	Gly	Lys	Ser	Leu 45	Glu	Trp	Ile	
Gly	Asp 50	Ile	Asn	Pro	Asn	Asn 55	Gly	Gly	Thr	Asp	Tyr 60	Asn	Gln	Asn	Phe	
Lys 65	Gly	Lys	Ala	Thr	Leu 70	Thr	Val	Asp	Lys	Ser 75	Ser	Ser	Thr	Ala	Tyr 80	
Met	Gln	Leu	Asn	Ser 85	Leu	Thr	Ser	Glu	Asp 90	Ser	Ala	Val	Tyr	Tyr 95	Cys	
Ala	Arg	Glu	Gly 100	Asn	Asn	Tyr	Gly	Ser 105	Arg	Asp	Ala	Met	Asp 110	Tyr	Trp	
Gly	Gln	Gly 115	Thr	Ser	Val	Thr	Val 120	Ser	Ser	Ala	Lys	Thr 125	Thr	Ala	Pro	
Ser	Val 130	Tyr	Pro	Leu	Ala	Pro 135	Val	Cys	Gly	Asp	Thr 140	Thr	Gly	Ser	Ser	
Val 145	Thr	Leu	Gly	Cys	Leu 150	Val	Lys	Gly	Tyr	Phe 155	Pro	Glu	Pro	Val	Thr 160	
Leu	Thr	Trp	Asn	Ser 165	Gly	Ser	Leu	Ser	Ser 170	Gly	Val	His	Thr	Phe 175	Pro	
Ala	Val	Leu	Gln 180	Ser	Asp	Leu	Tyr	Thr 185	Leu	Ser	Ser	Ser	Val 190	Thr	Val	
Thr	Ser	Ser 195	Thr	Trp	Pro	Ser	Gln 200	Ser	Ile	Thr	Cys	Asn 205	Val	Ala	His	
Pro	Ala 210	Ser	Ser	Thr	Lys	Val 215	Asp	Lys	Lys	Ile	Glu 220	Pro	Arg	Gly	Pro	
Thr 225	Ile	Lys	Pro	Cys	Pro 230	Pro	Cys	Lys	Cys	Pro 235	Ala	Pro	Asn	Leu	Leu 240	
Gly	Gly	Pro	Ser	Val 245	Phe	Ile	Phe	Pro	Pro 250	Lys	Ile	Lys	Asp	Val 255	Leu	
Met	Ile	Ser	Leu 260	Ser	Pro	Ile	Val	Thr 265	Cys	Val	Val	Val	Asp 270	Val	Ser	
Glu	Asp	Asp 275	Pro	Asp	Val	Gln	Ile 280	Ser	Trp	Phe	Val	Asn 285	Val	Glu		

735042006540SeqList.txt

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Val  His  Thr  Ala  Gln  Thr  Gln  Thr  His  Arg  Glu  Asp  Tyr  Asn  Ser  Thr
 290          295          300
Leu  Arg  Val  Val  Ser  Ala  Leu  Pro  Ile  Gln  His  Gln  Asp  Trp  Met  Ser
305          310          315
Gly  Lys  Glu  Phe  Lys  Cys  Lys  Val  Asn  Asn  Lys  Asp  Leu  Pro  Ala  Pro
          325          330          335
Ile  Glu  Arg  Thr  Ile  Ser  Lys  Pro  Lys  Gly  Ser  Val  Arg  Ala  Pro  Gln
          340          345          350
Val  Tyr  Val  Leu  Pro  Pro  Pro  Glu  Glu  Glu  Met  Thr  Lys  Lys  Gln  Val
          355          360          365
Thr  Leu  Thr  Cys  Met  Val  Thr  Asp  Phe  Met  Pro  Glu  Asp  Ile  Tyr  Val
          370          375          380
Glu  Trp  Thr  Asn  Asn  Gly  Lys  Thr  Glu  Leu  Asn  Tyr  Lys  Asn  Thr  Glu
385          390          395          400
Pro  Val  Leu  Asp  Ser  Asp  Gly  Ser  Tyr  Phe  Met  Tyr  Ser  Lys  Leu  Arg
          405          410          415
Val  Glu  Lys  Lys  Asn  Trp  Val  Glu  Arg  Asn  Ser  Tyr  Ser  Cys  Ser  Val
          420          425          430
Val  His  Glu  Gly  Leu  His  Asn  His  His  Thr  Thr  Lys  Ser  Phe  Ser  Arg
          435          440          445
Thr  Pro  Gly  Lys
          450

```

<210> 39
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-1 HC signal sequence

<400> 39
 Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15
 Val Leu Ser

<210> 40
 <211> 106
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-1 VL

<400> 40
 Gln Ile Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Pro Gly
 1 5 10 15
 Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Gly Val Ile Tyr Met
 20 25 30
 Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
 35 40 45
 Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
 65 70 75 80
 Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
 85 90 95
 Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 100 105

<210> 41
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>

<223> anti-ID B-1 CL

<400> 41

```

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
1      5      10      15
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
20      25      30
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
35      40      45
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
50      55      60
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
65      70      75      80
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
85      90      95
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
100      105

```

<210> 42

<211> 213

<212> PRT

<213> Artificial Sequence

<220>

<223> anti-ID B-1 light chain

<400> 42

```

Gln Ile Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Pro Gly
1      5      10      15
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Gly Val Ile Tyr Met
20      25      30
Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
35      40      45
Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50      55      60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65      70      75      80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85      90      95
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro
100      105      110
Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly
115      120      125
Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn
130      135      140
Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn
145      150      155      160
Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser
165      170      175
Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr
180      185      190
Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe
195      200      205
Asn Arg Asn Glu Cys
210

```

<210> 43

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> anti-ID B-1 LC signal sequence

735042006540SeqList.txt

<400> 43
Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Met Ser Ala Ser
1 5 10 15
Val Ile Met Ser Arg Gly
20

<210> 44
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID B-1 HC-CDR1

<400> 44
Asp Tyr Tyr Met Lys
1 5

<210> 45
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID B-1 HC-CDR2

<400> 45
Asp Ile Asn Pro Asn Asn Gly Gly Thr Asp Tyr Asn Gln Asn Phe Lys
1 5 10 15
Gly

<210> 46
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID B-1 HC-CDR3

<400> 46
Glu Gly Asn Asn Tyr Gly Ser Arg Asp Ala Met Asp Tyr
1 5 10

<210> 47
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID B-1 LC-CDR1

<400> 47
Ser Ala Ser Ser Gly Val Ile Tyr Met Tyr
1 5 10

<210> 48
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID B-1 LC-CDR2

<400> 48

Leu Thr Ser Asn Leu Ala Ser
1 5

<210> 49

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> anti-ID B-1 LC-CDR3

<400> 49

Gln Gln Trp Ser Ser Asn Pro Leu Thr
1 5

<210> 50

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-1 VH

<400> 50

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catggaaaga	gccttgagtg	gattggagat	attaatccta	acaatgggtg	tactgactac	180
aaccagaact	ttaagggcaa	ggccacattg	actgtagaca	aatcctccag	cacagcctac	240
atgcagctca	acagcctgac	atctgaggac	tctgcagtct	attactgtgc	aagagagggg	300
aataactacg	gtagtagaga	tgctatggac	tactggggtc	aaggaacgtc	agtcaccgtc	360
tcctca						366

<210> 51

<211> 990

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-1 CH

<400> 51

gccaaaacaa	cagccccatc	ggtctatcca	ctggccccctg	tgtgtggaga	tacaactggc	60
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tggaaactctg	gatccctgtc	cagtgggtgtg	cacaccttcc	cagctgtcct	gcagtctgac	180
ctctacaccc	tcagcagctc	agtgactgta	acctcgagca	cctggcccag	ccagtcctac	240
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gggcccacaa	tcaagccctg	tcctccatgc	aaatgcccag	cacctaacct	cttgggtgga	360
ccatccgtct	tcattcttccc	tccaaagatc	aaggatgtac	tcatgatctc	cctgagcccc	420
atagtcacat	gtgtgggtgt	ggatgtgagc	gaggatgacc	cagatgtcca	gatcagctgg	480
tttgtgaaca	acgtggaagt	acacacagct	cagacacaaa	cccatagaga	ggattacaac	540
agtactctcc	gggtgggtcag	tgccctcccc	atccagcacc	aggactggat	gagtggcaag	600
gagttcaaat	gcaagggtcaa	caacaaagac	ctcccagcgc	ccatcgagag	aaccatctca	660
aaacccaaag	ggtcagtaag	agctccacag	gtatatgtct	tgccctccacc	agaagaagag	720
atgactaaga	aacagggtcac	tctgacctgc	atgggtcacag	acttcatgcc	tgaagacatt	780
tacgtggagt	ggaccaacaa	cgggaaaaca	gagctaaact	acaagaacac	tgaaccagtc	840
ctggactctg	atggttctta	cttcatgtac	agcaagctga	gagtggaaaa	gaagaactgg	900
gtggaagaaa	atagctactc	ctgttcagtg	gtccacgagg	gtctgcacaa	tcaccacacg	960
actaagagct	tctcccggac	tccgggttaa				990

<210> 52

<211> 1356

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-1 heavy chain

<400> 52

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catggaaaga	gccttgagtg	gattggagat	attaatccta	acaatgggtg	tactgactac	180
aaccagaact	ttaagggcaa	ggccacattg	actgtagaca	aatcctccag	cacagcctac	240
atgcagctca	acagcctgac	atctgaggac	tctgcagtct	attactgtgc	aagagagggg	300
aataactacg	gtagtagaga	tgctatggac	tactggggtc	aaggaacgtc	agtcaccgtc	360
tcctcagcca	aaacaacagc	cccatcggtc	tatccactgg	cccctgtgtg	tggagatata	420
actggctcct	cggtgactct	aggatgcctg	gtcaagggtt	atctccctga	gccagtgacc	480
ttgacctgga	actctggatc	cctgtccagt	ggtgtgcaca	ccttcccagc	tgtcctgcag	540
tctgacctct	acaccctcag	cagctcagtg	actgtaacct	cgagcacctg	gcccagccag	600
tcctcacctt	gcaatgtggc	ccaccgggca	agcagcacca	aggtggacaa	gaaaattgag	660
cccagagggc	ccacaatcaa	gccctgtcct	ccatgcaaat	gcccagcacc	taacctcttg	720
ggtggaccat	ccgtcttcat	cttccctcca	aagatcaagg	atgtactcat	gatctccctg	780
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ggcaaggagt	tcaaatgcaa	ggtaacaac	aaagacctcc	cagcgcccat	cgagagaacc	1020
atctcaaaac	ccaaagggtc	agtaagagct	ccacaggtat	atgtcttgcc	tccaccagaa	1080
gaagagatga	ctaagaaaca	ggtcactctg	acctgcatgg	tcacagactt	catgcctgaa	1140
gacatttacg	tggagtggac	caacaacggg	aaaacagagc	taaactacaa	gaacactgaa	1200
ccagtccttg	actctgatgg	ttcttacttc	atgtacagca	agctgagagt	ggaaaagaag	1260
aactgggtgg	aaagaaatag	ctactcctgt	tcagtgggtc	acgaggggtc	gcacaatcac	1320
cacacgacta	agagcttctc	ccggactccg	ggtaaa			1356

<210> 53

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-1 HC signal sequence

<400> 53

atgggatgga	gctggatctt	tctcttcctc	ttgtcaggaa	ctgcaggtgt	cctctct	57

<210> 54

<211> 318

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-1 VL

<400> 54

caaattgttc	tcaccagtc	tccagcactc	atgtctgcat	ctccagggga	gaaggtcacc	60
atgacctgca	gtgccagctc	aggtgtaatt	tacatgtact	ggtaccaaca	gaagccaaga	120
tcctccccc	aaccctggat	ttatctcaca	tccaacctgg	cttctggagt	ccctgctcgc	180
ttcagtggca	gtgggtctgg	gacctcttac	tctctcacia	tcagcagcat	ggaggctgaa	240
gatgctgcca	cttattactg	ccagcagtgg	agtagtaacc	cgctcacgtt	cggtgctggc	300
accaagctgg	agctgaaa					318

<210> 55

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-1 CL

<400> 55

cgggctgatg	ctgcaccaac	tgtatccatc	ttcccaccat	ccagttagca	gttaacatct	60
ggaggtgcc	cagtcgtgtg	cttcttgaac	aacttctacc	ccaaagacat	caatgtcaag	120
tggaagattg	atggcagtga	acgacaaaat	ggcgctcctga	acagttggac	tgatcaggac	180
agcaaagaca	gcacctacag	catgagcagc	accctcacgt	tgaccaagga	cgagtatgaa	240

735042006540seqList.txt

cgacataaca gctatacctg tgaggccact cacaagacat caacttcacc cattgtcaag 300
 agcttcaaca ggaatgagtg t 321

<210> 56
 <211> 639
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID B-1 light chain

<400> 56
 caaattgttc tcaccagtc tccagcactc atgtctgcat ctccagggga gaaggtcacc 60
 atgacctgca gtgccagctc aggtgtaatt tacatgtact ggtaccaaca gaagccaaga 120
 tcctcccca aaccctggat ttatctcaca tccaacctgg cttctggagt ccctgctcgc 180
 ttcagtggca gtgggtctgg gacctcttac tctctcaca tcagcagcat ggaggctgaa 240
 gatgctgcca cttattactg ccagcagtggt agtagtaacc cgctcacgtt cggtgctggc 300
 accaagctgg agctgaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc 360
 agtgagcagt taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc 420
 aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac 480
 agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcacgttg 540
 accaaggacg agtatgaacg acataacagc tatacctgtg aggccactca caagacatca 600
 acttcacca ttgtcaagag cttcaacagc aatgagtg 639

<210> 57
 <211> 66
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> anti-ID B-1 LC signal sequence

<400> 57
 atggattttc aagtgcagat tttcagcttc ctgctaata ga gtcctcagt cataatgtcc 60
 agggga 66

<210> 58
 <211> 115
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-2 VH

<400> 58
 Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ala
 1 5 10 15
 Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Phe Thr Arg Tyr
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Leu Asn Gln Lys Phe
 50 55 60
 Lys Asp Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 Ala Ser Ile Tyr Tyr Glu Glu Ala Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val Ser Ala
 115

<210> 59
 <211> 324
 <212> PRT
 <213> Artificial Sequence

<220>

<223> anti-ID B-2 CH

<400> 59

Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
 1 5 10 15
 Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
 20 25 30
 Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser
 35 40 45
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
 50 55 60
 Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
 65 70 75 80
 Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
 85 90 95
 Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
 100 105 110
 Glu Val Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
 115 120 125
 Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
 130 135 140
 Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
 145 150 155 160
 Val His Thr Ala Gln Thr Gln Pro Arg Glu Gln Phe Asn Ser Thr
 165 170 175
 Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
 180 185 190
 Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
 195 200 205
 Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
 210 215 220
 Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
 225 230 235 240
 Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
 245 250 255
 Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
 260 265 270
 Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
 275 280 285
 Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
 290 295 300
 Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
 305 310 315 320
 Ser Pro Gly Lys

<210> 60

<211> 439

<212> PRT

<213> Artificial Sequence

<220>

<223> anti-ID B-2 heavy chain

<400> 60

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ala
 1 5 10 15
 Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Phe Thr Arg Tyr
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Leu Asn Gln Lys Phe
 50 55 60
 Lys Asp Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys

<223> anti-ID B-2 VL

<400> 62

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Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
 1      5      10      15
Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
 20      25      30
Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Val
 35      40      45
Tyr Asn Ala Lys Thr Leu Ala Asp Ser Val Pro Ser Arg Phe Ser Gly
 50      55      60
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro
 65      70      75      80
Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Tyr
 85      90      95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100      105

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<210> 63

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> anti-ID B-2 light chain

<400> 63

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Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
 1      5      10      15
Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
 20      25      30
Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
 35      40      45
Tyr Asn Ala Lys Thr Leu Ala Asp Ser Val Pro Ser Arg Phe Ser Gly
 50      55      60
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro
 65      70      75      80
Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Tyr
 85      90      95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala
100      105      110
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115      120      125
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
130      135      140
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145      150      155      160
Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
165      170      175
Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
180      185      190
Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
195      200      205
Phe Asn Arg Asn Glu Cys
210

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<210> 64

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> anti-ID B-2 LC signal sequence

<400> 64

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Met Ser Val Leu Thr Gln Val Leu Ala Leu Leu Leu Leu Trp Leu Thr

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1 5 10 15
 Gly Ala Arg Cys
 20

<210> 65
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-2 HC-CDR1

<400> 65
 Arg Tyr Trp Met Asn
 1 5

<210> 66
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-2 HC-CDR2

<400> 66
 Met Ile His Pro Ser Asp Ser Glu Thr Arg Leu Asn Gln Lys Phe Lys
 1 5 10 15
 Asp

<210> 67
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-2 HC-CDR3

<400> 67
 Ile Tyr Tyr Glu Glu Ala
 1 5

<210> 68
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-2 LC-CDR1

<400> 68
 Arg Ala Ser Gly Asn Ile His Asn Tyr Leu Ala
 1 5 10

<210> 69
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> anti-ID B-2 LC-CDR2

<400> 69

Asn Ala Lys Thr Leu Ala Asp
1 5

<210> 70
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-ID B-2 LC-CDR3

<400> 70
Gln His Phe Trp Ser Thr Pro Tyr Thr
1 5

<210> 71
<211> 345
<212> DNA
<213> Artificial Sequence

<220>
<223> anti-ID B-2 VH

<400> 71
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tcctgcaaga cttctggcta ctcccttcacc aggtactgga tgaactgggt gaagcagagg 120
cctggacaag gccttgagt gattggcatg attcatcctt ccgatagtga aactagggtta 180
aatcagaagt tcaaggacaa ggccacattg actgtagaca attcctccag cacagcctac 240
atgcaactca gcagcccgcac atctgaggac tctgcggtct attactgtgc aagcatctac 300
tatgaagagg cctggggcca agggactctg gtcactgtct ctgca 345

<210> 72
<211> 972
<212> DNA
<213> Artificial Sequence

<220>
<223> anti-ID B-2 CH

<400> 72
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tggaactctg gatccctgtc cagcgggtgtg cacaccttcc cagctgtcct gcagtctgac 180
ctctacactc tgagcagctc agtgactgtc cctccagca cctggcccag cgagaccgtc 240
acctgcaacg ttgcccaccc ggccagcagc accaagggtg acaagaaaat tgtgcccagg 300
gattgtgggt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc 360
ccccaaagc ccaaggatgt gctcaccatt actctgactc ctaagggtcac gtgtgtttgtg 420
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agtctgacct gcatgataac agacttcttc cctgaagaca ttactgtgga gtggcagtgg 780
aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct 840
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acctgtcttg tttacatga gggcctgcac aaccaccata ctgagaagag cctctccac 960
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<210> 73
<211> 1317
<212> DNA
<213> Artificial Sequence

<220>
<223> anti-ID B-2 heavy chain

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<400> 73

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caggtccaac tgcagcagcc tggggctgag ctgggtgaggc ctggagcttc agtgaagctg 60
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cctggacaag gccttgagtg gattggcatg attcatcctt ccgatatgta aactaggtta 180
aatcagaagt tcaaggacaa ggccacattg actgtagaca attcctccag cacagcctac 240
atgcaactca gcagcccgc atctgaggac tctgcggtct attactgtgc aagcatctac 300
tatgaagagg cctggggcca agggactctg gtcactgtct ctgcagccaa aacgacaccc 360
ccatctgtct atccactggc ccctggatct gctgccc aaa ctaactccat ggtgaccctg 420
ggatgcctgg tcaagggcta tttccctgag ccagtgcag tgacctggaa ctctggatcc 480
ctgtccagcg gtgtgcacac cttcccagct gtcctgcagt ctgacctcta cactctgagc 540
agctcagtga ctgtccctc cagcacctgg cccagcgaga ccgtcacctg caacgttgcc 600
caccggcca gcagaccaa ggtggacaag aaaattgtgc ccagggattg tggttgtaag 660
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gatgtgtcga ccattactct gactcctaag gtcacgtgtg ttgtggtaga catcagcaag 780
gatgatcccg aggtccagtt cagctggttt gtagatgatg tggaggtgca cacagctcag 840
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tacaccattc cactcccaa ggagcagatg gccaaaggata aagtcagtct gacctgcatg 1080
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<210> 74

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-2 HC signal sequence

<400> 74

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atgggatgga gctctatcat cctcttcttg gtagcaacag ctacaggtgt ccactcc 57

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<210> 75

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-2 VL

<400> 75

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atcacatgtc gagcaagtgg gaatattcac aattatttag catggtatca gcagaaacag 120
ggaaaatctc ctgagctcct ggtctataat gcaaaaacct tagcagatag tgtgccatca 180
aggttcagtg gcagtggatc aggaacacaa tattctctca agatcaacag cctgcagcct 240
gaagattttg ggagttatta ctgtcaacat ttttgagta ctccgtacac gttcggaggg 300
gggaccaagc tggaaataaa a

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<210> 76

<211> 642

<212> DNA

<213> Artificial Sequence

<220>

<223> anti-ID B-2 light chain

<400> 76

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gacatccaga tgactcagtc tccagcctcc ctatctgcat ctgtgggaga aactgtcacc 60
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ggaaaatctc ctgagctcct ggtctataat gcaaaaacct tagcagatag tgtgccatca 180
aggttcagtg gcagtggatc aggaacacaa tattctctca agatcaacag cctgcagcct 240
gaagattttg ggagttatta ctgtcaacat ttttgagta ctccgtacac gttcggaggg 300
gggaccaagc tggaaataaa acgggctgat gctgcacaa ctgtatccat cttcccacca 360
tcagtgagc agttaacatc tggaggtgcc gcttcttgaa caacttctac 420
cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgctcctg 480

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<400> 78
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 1 5

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 Thr Ser Tyr Trp Met His
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 Tyr Pro Gly Ser Gly Gly
 1 5

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<220>
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 Asn Ile Tyr Pro Gly Ser Gly Gly Thr Asn
 1 5 10

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<400> 83
 Trp Ile Gly Asn Ile Tyr Pro Gly Ser Gly Gly Thr Asn
 1 5 10

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<400> 84
 Thr Arg Glu Val Thr Thr Val Ala Tyr Tyr Tyr Ser Met Asp
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<400> 85
 Ser Asn Tyr Leu Asn Trp Tyr
 1 5

<210> 86
 <211> 10
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<400> 86
 Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His
 1 5 10

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<213> Artificial Sequence

<220>

<223> anti-ID LC-CDR3

<400> 87

Gln Gln Gly Lys Thr Val Pro Phe
1 5

<210> 88

<211> 7

<212> PRT

<213> anti-ID B-1 HC-CDR1

<400> 88

Gly Tyr Thr Phe Thr Asp Tyr
1 5

<210> 89

<211> 10

<212> PRT

<213> Artificial Sequence

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Gly Tyr Thr Phe Thr Asp Tyr Tyr Met Lys
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<211> 6

<212> PRT

<213> Artificial Sequence

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Thr Asp Tyr Tyr Met Lys
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Asn Pro Asn Asn Gly Gly
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Asp Ile Asn Pro Asn Asn Gly Gly Thr Asp
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 Trp Ile Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Asp
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 Ala Arg Glu Gly Asn Asn Tyr Gly Ser Arg Asp Ala Met Asp
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 Ile Tyr Met Tyr Trp Tyr
 1 5

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<400> 96
 Pro Trp Ile Tyr Leu Thr Ser Asn Leu Ala
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 Gln Gln Trp Ser Ser Asn Pro Leu
 1 5

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<220>
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<400> 98
 Gly Tyr Ser Phe Thr Arg Tyr
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 Gly Tyr Ser Phe Thr Arg Tyr Trp Met Asn
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 Thr Arg Tyr Trp Met Asn
 1 5

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 His Pro Ser Asp Ser Glu
 1 5

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 Met Ile His Pro Ser Asp Ser Glu Thr Arg
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 Trp Ile Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg
 1 5 10

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<400> 105
 His Asn Tyr Leu Ala Trp Tyr
 1 5

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<220>
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<400> 106
 Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala
 1 5 10

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<400> 107
 Gln His Phe Trp Ser Thr Pro Tyr
 1 5

<210> 108
 <211> 10
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 <213> Artificial Sequence


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<220>
<223> HC-CDR1 Consensus

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<223> Xaa = T or S

<220>
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<220>
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<222> 6
<223> Xaa = D or R

<220>
<221> VARIANT
<222> 8
<223> Xaa = Y or W

<220>
<221> VARIANT
<222> 10
<223> Xaa = K or N

<400> 108
Gly Tyr Xaa Phe Xaa Xaa Tyr Xaa Met Xaa
 1              5              10

<210> 109
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> HC-CDR2 Consensus

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<223> Xaa = D or M

<220>
<221> VARIANT
<222> 6
<223> Xaa = N or H

<220>
<221> VARIANT
<222> 8
<223> Xaa = N or S

<220>
<221> VARIANT
<222> 9
<223> Xaa = N or D

<220>
<221> VARIANT
<222> 10
<223> Xaa = G or S

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<220>
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 <222> (11)...(11)
 <223> Xaa = G or E

<220>
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<220>
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 <222> (17)...(17)
 <223> Xaa = N or K

<220>
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 <223> Xaa = G or D

<400> 109
 Trp Ile Gly Xaa Ile Xaa Pro Xaa Xaa Xaa Xaa Thr Xaa Xaa Asn Gln
 1 5 10 15
 Xaa Phe Lys Xaa
 20

<210> 110
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HC-CDR3 Consensus

<220>
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 <223> Xaa = R or S

<220>
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 <222> 3
 <223> Xaa = E or I

<220>
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 <222> 4
 <223> Xaa = G or Y

<220>
 <221> VARIANT
 <222> 5
 <223> Xaa = N or Y

<220>
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<222> 6
 <223> Xaa = N or E

<220>
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 <222> (8)...(8)
 <223> Xaa = G or null

<220>
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 <222> (9)...(9)
 <223> Xaa = S or null

<220>
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 <222> (10)...(10)
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<220>
 <221> VARIANT
 <222> (11)...(11)
 <223> Xaa = D or null

<220>
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 <222> (12)...(12)
 <223> Xaa = A or null

<220>
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 <222> (13)...(13)
 <223> X13 = M or null

<220>
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 <222> (14)...(14)
 <223> X14 = D or E

<220>
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 <222> (15)...(15)
 <223> X15 = Y or A

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 1 5 10 15

<210> 111
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<220>

<223> LC-CDR1 Consensus

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<223> Xaa = S or R

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<223> Xaa = S or G

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<223> Xaa = I or H

<220>

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<223> Xaa = N or null

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<222> (10)...(10)

<223> Xaa = M or L

<220>

<221> VARIANT

<222> (11)...(11)

<223> Xaa = Y or A

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Xaa Ala Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Trp Tyr
1 5 10

<210> 112

<211> 11

<212> PRT

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<220>

<223> LC-CDR2 Consensus

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<223> Xaa = W or L

<220>

<221> VARIANT

<222> (3)...(3)

<223> Xaa = I or V

<220>

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<222> (5)...(5)

<223> Xaa = L or N

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<221> VARIANT

<222> (6)...(6)

<223> Xaa = T or A

<220>

<221> VARIANT

<222> (7)...(7)

<223> Xaa = S or K

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<221> VARIANT

<222> (8)...(8)

<223> Xaa = N or T

<220>

<221> VARIANT

<222> (11)...(11)

<223> Xaa = S or D

<400> 112

Xaa Xaa Xaa Tyr Xaa Xaa Xaa Xaa Leu Ala Xaa
1 5 10

<210> 113

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> LC-CDR3 Consensus

<220>

<221> VARIANT

<222> (2)...(2)

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<221> VARIANT

<222> (3)...(3)

<223> Xaa =W or F

<220>

<221> VARIANT

<222> (4)...(4)

<223> Xaa = S or W

<220>
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 <223> Xaa = N or T

<220>
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 <222> (8)...(8)
 <223> Xaa = L or Y

<400> 113
 Gln Xaa Xaa Xaa Xaa Xaa Pro Xaa Thr
 1 5

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 <211> 5
 <212> PRT
 <213> Mus musculus

<220>
 <223> SJ25C1 HC-CDR1

<400> 114
 Ser Tyr Trp Met Asn
 1 5

<210> 115
 <211> 17
 <212> PRT
 <213> Mus musculus

<220>
 <223> SJ25C1 HC-CDR2

<400> 115
 Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
 1 5 10 15
 Gly

<210> 116
 <211> 13
 <212> PRT
 <213> Mus musculus

<220>
 <223> SJ25C1 HC-CDR3

<400> 116
 Lys Thr Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Tyr
 1 5 10

<210> 117
 <211> 11
 <212> PRT
 <213> Mus musculus

<220>

<223> SJ25C1 LC-CDR1

<400> 117

Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala
 1 5 10

<210> 118

<211> 7

<212> PRT

<213> Mus musculus

<220>

<223> SJ25C1 LC-CDR2

<400> 118

Ser Ala Thr Tyr Arg Asn Ser
 1 5

<210> 119

<211> 9

<212> PRT

<213> Mus musculus

<220>

<223> SJ25C1 LC-CDR3

<400> 119

Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr
 1 5

<210> 120

<211> 5

<212> PRT

<213> Mus musculus

<220>

<223> SJ25C1 LC-CDR3

<400> 120

Asp Tyr Gly Val Ser
 1 5

<210> 121

<211> 16

<212> PRT

<213> Mus musculus

<220>

<223> FMC63 HC-CDR2

<400> 121

Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser
 1 5 10 15

<210> 122

<211> 12

<212> PRT

<213> Mus musculus

<220>

<223> FMC63 HC-CDR3

<400> 122
 His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
 1 5 10

<210> 123
 <211> 11
 <212> PRT
 <213> Mus musculus

<220>
 <223> FMC63 LC-CDR1

<400> 123
 Arg Ala Ser Gln Asp Ile Ser Lys Tyr Leu Asn
 1 5 10

<210> 124
 <211> 7
 <212> PRT
 <213> Mus musculus

<220>
 <223> FMC63 LC-CDR2

<400> 124
 His Thr Ser Arg Leu His Ser
 1 5

<210> 125
 <211> 9
 <212> PRT
 <213> Mus musculus

<220>
 <223> FMC63 LC-CDR3

<400> 125
 Gln Gln Gly Asn Thr Leu Pro Tyr Thr
 1 5

<210> 126
 <211> 24
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> T2A

<400> 126
 Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
 1 5 10 15
 Val Glu Glu Asn Pro Gly Pro Arg
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<210> 127
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> T2A

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<400> 127

Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro
 1 5 10 15
 Gly Pro

<210> 128

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> P2A

<400> 128

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val
 1 5 10 15
 Glu Glu Asn Pro Gly Pro
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<210> 129

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> P2A

<400> 129

Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn
 1 5 10 15
 Pro Gly Pro

<210> 130

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> E2A

<400> 130

Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser
 1 5 10 15
 Asn Pro Gly Pro
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<210> 131

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> F2A

<400> 131

Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val
 1 5 10 15
 Glu Ser Asn Pro Gly Pro
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<210> 132

<211> 459

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary humanized anti-SJ25C1 heavy chain

<400> 132

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Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1      5      10      15
Ser Leu Ser Ile Thr Cys Thr Val Ser Asp Tyr Thr Phe Thr Ser Tyr
      20      25      30
Gly Val His Trp Val Arg Gln Ser Gly Lys Gly Leu Glu Trp Leu
      35      40      45
Gly Val Ile Tyr Pro Gly Ser Gly Gly Thr Asp Tyr Asn Thr Pro Phe
      50      55      60
Thr Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe
65      70      75      80
Phe Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys
      85      90      95
Ala Arg Glu Val Thr Thr Val Ala Tyr Tyr Tyr Ser Met Asp Tyr Trp
      100      105      110
Gly Gln Gly Thr Leu Val Thr Val Ser Ala Thr Leu Val Thr Val Ser
      115      120      125
Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
130      135      140
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
145      150      155      160
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
      165      170      175
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
      180      185      190
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
      195      200      205
Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
210      215      220
Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
225      230      235      240
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
      245      250      255
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
      260      265      270
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
      275      280      285
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
290      295      300
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305      310      315      320
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
      325      330      335
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
      340      345      350
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
      355      360      365
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
370      375      380
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
385      390      395      400
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
      405      410      415
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
      420      425      430
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
435      440      445
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450      455

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<210> 133
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-SJ25C1 light chain

<400> 133
 Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
 1 5 10 15
 Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
 35 40 45
 Lys Tyr Thr Ser Arg Leu His Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
 65 70 75 80
 Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Gly Lys Thr Val Pro Phe
 85 90 95
 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> 134
 <211> 459
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-SJ25C1 heavy chain

<400> 134
 Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
 20 25 30
 Trp Met His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Asn Ile Tyr Pro Gly Ser Gly Gly Thr Asn Tyr Asn Thr Pro Phe
 50 55 60
 Thr Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe
 65 70 75 80
 Phe Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 Thr Arg Glu Val Thr Thr Val Ala Tyr Tyr Ser Met Asp Tyr Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ala Thr Leu Val Thr Val Ser
 115 120 125
 Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
 130 135 140
 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
 145 150 155 160

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Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
 165 170 175
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
 180 185 190
 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
 195 200 205
 Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
 210 215 220
 Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 225 230 235 240
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 245 250 255
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 260 265 270
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 275 280 285
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 290 295 300
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 305 310 315 320
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 325 330 335
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 340 345 350
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 355 360 365
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 370 375 380
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 385 390 395 400
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 405 410 415
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 420 425 430
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455

<210> 135

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary humanized anti-SJ25C1 light chain

<400> 135

Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
 1 5 10 15
 Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
 35 40 45
 Tyr Tyr Thr Ser Arg Leu His Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
 65 70 75 80
 Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Gly Lys Thr Val Pro Phe
 85 90 95
 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln

[illegible]

<220>
<223> Exemplary humanized anti-SJ25C1 heavy chain

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Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 385 390 395 400
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 405 410 415
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 420 425 430
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455

<210> 137
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-SJ25C1 light chain

<400> 137
 Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
 1 5 10 15
 Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
 35 40 45
 Lys Tyr Thr Ser Arg Leu His Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
 65 70 75 80
 Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Gly Lys Thr Val Pro Phe
 85 90 95
 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> 138
 <211> 459
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-FMC63 heavy chain

<400> 138
 Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30
 Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Val Ile Asn Pro Asn Asn Gly Gly Thr Asp Tyr Asn Thr Pro Phe
 50 55 60

735042006540SeqList.txt

Thr	Ser	Arg	Leu	Ser	Ile	Asn	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe
65					70					75					80
Phe	Lys	Met	Asn	Ser	Leu	Gln	Ser	Asn	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Glu	Gly	Asn	Asn	Tyr	Gly	Ser	Arg	Asp	Ala	Met	Asp	Tyr	Trp
			100					105					110		
Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Thr	Leu	Val	Thr	Val	Ser
		115					120					125			
Ala	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser
	130					135					140				
Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp
145				150						155					160
Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr
				165					170					175	
Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr
			180					185					190		
Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln
		195					200					205			
Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp
	210					215					220				
Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro
225				230					235						240
Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro
				245					250					255	
Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr
			260					265					270		
Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn
		275					280					285			
Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg
	290					295					300				
Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val
305				310						315					320
Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser
				325					330					335	
Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys
			340					345					350		
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp
		355					360					365			
Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe
	370					375					380				
Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu
385				390					395						400
Asn	Asn	Tyr	Lys	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Gly	Phe
				405				410					415		
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly
			420					425					430		
Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr
		435					440					445			
Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys					
	450					455									

<210> 139

<211> 213

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary humanized anti-FMC63 light chain

<400> 139

Asp	Ile	Leu	Leu	Thr	Gln	Ser	Pro	Val	Ile	Leu	Ser	Val	Ser	Pro	Gly
1				5				10					15		
Glu	Arg	Val	Ser	Phe	Ser	Cys	Ser	Ala	Ser	Ser	Gly	Val	Ile	Tyr	Met
			20					25				30			
Tyr	Trp	Tyr	Gln	Gln	Arg	Thr	Asn	Gly	Ser	Pro	Arg	Leu	Leu	Ile	Lys
		35					40					45			
Leu	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser

[illegible]

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<210> 140
<211> 459
<212> PRT
<213> Artificial Sequence
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<220>
<223> Exemplary humanized anti-FMC63 heavy chain

<400>	140														
Gln	Val	Gln	Leu	Lys	Gln	Ser	Gly	Pro	Gly	Leu	Val	Gln	Pro	Ser	Gln
1				5					10					15	
Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Asp	Tyr
			20					25					30		
Tyr	Met	Lys	Trp	Val	Arg	Gln	Ser	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu
		35					40					45			
Gly	Asp	Ile	Asn	Pro	Asn	Asn	Gly	Gly	Thr	Asp	Tyr	Asn	Gln	Asn	Phe
	50					55					60				
Lys	Gly	Arg	Leu	Ser	Ile	Asn	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe
	65				70					75					80
Phe	Lys	Met	Asn	Ser	Leu	Gln	Ser	Asn	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Glu	Gly	Asn	Asn	Tyr	Gly	Ser	Arg	Asp	Ala	Met	Asp	Tyr	Trp
			100					105					110		
Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Thr	Leu	Val	Thr	Val	Ser
		115					120					125			
Ala	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser
	130					135					140				
Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp
	145				150					155					160
Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr
				165					170					175	
Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr
			180					185						190	
Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln
		195					200					205			
Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp
	210					215					220				
Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro
	225				230					235					240
Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro
				245					250					255	
Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr
			260					265					270		
Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn
		275					280					285			

735042006540seqList.txt

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Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
290 300
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305 310 315 320
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
325 330 335
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
340 345 350
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
355 360 365
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
370 375 380
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
385 390 395 400
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
405 410 415
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
420 425 430
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
435 440 445
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455

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<210> 141
 <211> 213
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-FMC63 light chain

```

<400> 141
Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Val Ser Phe Ser Cys Ser Ala Ser Ser Gly Val Ile Tyr Met
20 25 30
Tyr Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile Lys
35 40 45
Leu Thr Ser Asn Leu Ala Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser
50 55 60
Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser Glu
65 70 75 80
Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro
100 105 110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205
Asn Arg Gly Glu Cys
210

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<210> 142
 <211> 459
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Exemplary humanized anti-FMC63 heavy chain

<400> 142

Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asp Tyr
 20 25 30
 Tyr Met Lys Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Asp Tyr Asn Thr Pro Phe
 50 55 60
 Thr Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe
 65 70 75 80
 Phe Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Gly Asn Asn Tyr Gly Ser Arg Asp Ala Met Asp Tyr Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ala Thr Leu Val Thr Val Ser
 115 120 125
 Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
 130 135 140
 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
 145 150 155 160
 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
 165 170 175
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
 180 185 190
 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
 195 200 205
 Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
 210 215 220
 Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 225 230 235 240
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 245 250 255
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 260 265 270
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 275 280 285
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 290 295 300
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 305 310 315 320
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 325 330 335
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 340 345 350
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 355 360 365
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 370 375 380
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 385 390 395 400
 Asn Asn Tyr Lys Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 405 410 415
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 420 425 430
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455

<210> 143

<211> 213

<212> PRT

<213> Artificial sequence

<220>

<223> Exemplary humanized anti-FMC63 light chain

<400> 143

```

Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
 1      5      10      15
Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Ile Tyr Met
 20      25      30
Tyr Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Pro Trp Ile Tyr
 35      40      45
Leu Thr Ser Asn Leu Ala Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser
 50      55      60
Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser Glu
 65      70      75      80
Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
 85      90      95
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro
 100     105     110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
 115     120     125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
 130     135     140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
 145     150     155     160
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165     170     175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
 180     185     190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
 195     200     205
Asn Arg Gly Glu Cys
 210

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<210> 144

<211> 452

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary humanized anti-FMC63 heavy chain

<400> 144

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Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1      5      10      15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Tyr Ser Phe Thr Arg Tyr
 20      25      30
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
 35      40      45
Gly Val Ile His Pro Ser Asp Ser Glu Thr Asp Tyr Asn Thr Pro Phe
 50      55      60
Thr Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe
 65      70      75      80
Phe Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys
 85      90      95
Ala Arg Ile Tyr Tyr Glu Glu Ala Trp Gly Gln Gly Thr Leu Val Thr
 100     105     110
Val Ser Ala Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro
 115     120     125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130     135     140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145     150     155     160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165     170     175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180     185     190

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Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn
		195					200					205			
His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser
	210					215					220				
Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu
225					230					235					240
Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
				245					250					255	
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
			260					265					270		
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu
		275					280					285			
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr
	290					295					300				
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn
305					310					315					320
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro
				325					330					335	
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln
			340					345					350		
Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val
		355					360					365			
Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val
	370					375					380				
Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro
385					390					395					400
Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr
				405					410					415	
Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val
			420					425					430		
Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu
		435					440					445			
Ser	Pro	Gly	Lys												
		450													

<210> 145

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary humanized anti-FMC63 light chain

<400> 145

Asp	Ile	Leu	Leu	Thr	Gln	Ser	Pro	Val	Ile	Leu	Ser	Val	Ser	Pro	Gly
1				5					10					15	
Glu	Arg	Val	Ser	Phe	Ser	Cys	Arg	Ala	Ser	Gly	Asn	Ile	His	Asn	Tyr
			20					25					30		
Leu	Ala	Trp	Tyr	Gln	Gln	Arg	Thr	Asn	Gly	Ser	Pro	Arg	Leu	Leu	Ile
		35					40					45			
Lys	Asn	Ala	Lys	Thr	Leu	Ala	Asp	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly
	50					55				60					
Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Ser
65					70					75				80	
Glu	Asp	Ile	Ala	Asp	Tyr	Tyr	Cys	Gln	His	Phe	Trp	Ser	Thr	Pro	Tyr
				85					90					95	
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	Thr	Val	Ala	Ala
			100					105					110		
Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly
		115					120					125			
Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
	130					135					140				
Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145					150					155					160
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
				165					170					175	
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr

735042006540SeqList.txt

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 180 185 190
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> 146
 <211> 452
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-FMC63 heavy chain

<400> 146
 Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Arg Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Leu Asn Gln Lys Phe
 50 55 60
 Lys Asp Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe
 65 70 75 80
 Phe Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 Ala Arg Ile Tyr Tyr Glu Glu Ala Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val Ser Ala Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415

735042006540SeqList.txt

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 420 425 430
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 435 440 445
 Ser Pro Gly Lys
 450

<210> 147
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-FMC63 light chain

<400> 147
 Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
 1 5 10 15
 Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
 20 25 30
 Leu Ala Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
 35 40 45
 Lys Asn Ala Lys Thr Leu Ala Asp Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
 65 70 75 80
 Glu Asp Ile Ala Asp Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Tyr
 85 90 95
 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> 148
 <211> 452
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Exemplary humanized anti-FMC63 heavy chain

<400> 148
 Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Arg Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Tyr Asn Thr Pro Phe
 50 55 60
 Thr Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe
 65 70 75 80
 Phe Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95

735042006540seqList.txt

Ala Ser Ile Tyr Tyr Glu Glu Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ala Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro
115 120 125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445
Ser Pro Gly Lys
450

<210> 149

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Exemplary humanized anti-FMC63 light chain

<400> 149

Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile His Asn Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Val
35 40 45
Tyr Asn Ala Lys Thr Leu Ala Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
65 70 75 80
Glu Asp Ile Ala Asp Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Tyr

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      85      90      95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
      100      105      110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
      115      120      125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
      130      135      140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
      145      150      155
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
      160      165      170
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
      175      180      185
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
      190      195      200
Phe Asn Arg Gly Glu Cys
      205      210

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<210> 150
 <211> 12
 <212> PRT
 <213> Homo sapiens

<220>
 <223> spacer (IgG4hinge) (aa)

<400> 150
 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro
 1 5 10

<210> 151
 <211> 36
 <212> DNA
 <213> Homo sapiens

<220>
 <223> spacer (IgG4hinge) (nt)

<400> 151
 gaatctaagt acggaccgcc ctgccccct tgcct

36

<210> 152
 <211> 119
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Hinge-CH3 spacer

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<400> 152
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Gly Gln Pro Arg
1 5 10 15
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
20 25 30
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
35 40 45
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
50 55 60
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
65 70 75 80
Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
85 90 95
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
100 105 110
Leu Ser Leu Ser Leu Gly Lys
115

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<210> 153
 <211> 229
 <212> PRT
 <213> Homo sapiens

<220>
 <223> Hinge-CH2-CH3

<400> 153
 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe
 1 5 10 15
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 20 25 30
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 35 40 45
 Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
 50 55 60
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
 65 70 75 80
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 85 90 95
 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
 100 105 110
 Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 115 120 125
 Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
 130 135 140
 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 145 150 155 160
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 165 170 175
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
 180 185 190
 Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
 195 200 205
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 210 215 220
 Leu Ser Leu Gly Lys
 225

<210> 154
 <211> 282
 <212> PRT
 <213> Homo sapiens

<220>
 <223> IgD-hinge-Fc

<400> 154
 Arg Trp Pro Glu Ser Pro Lys Ala Gln Ala Ser Ser Val Pro Thr Ala
 1 5 10 15
 Gln Pro Gln Ala Glu Gly Ser Leu Ala Lys Ala Thr Thr Ala Pro Ala
 20 25 30
 Thr Thr Arg Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys
 35 40 45
 Glu Lys Glu Glu Gln Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro
 50 55 60
 Ser His Thr Gln Pro Leu Gly Val Tyr Leu Leu Thr Pro Ala Val Gln
 65 70 75 80
 Asp Leu Trp Leu Arg Asp Lys Ala Thr Phe Thr Cys Phe Val Val Gly
 85 90 95
 Ser Asp Leu Lys Asp Ala His Leu Thr Trp Glu Val Ala Gly Lys Val
 100 105 110
 Pro Thr Gly Gly Val Glu Glu Gly Leu Leu Glu Arg His Ser Asn Gly
 115 120 125

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Ser	Gln	Ser	Gln	His	Ser	Arg	Leu	Thr	Leu	Pro	Arg	Ser	Leu	Trp	Asn
Ala	Gly	Thr	Ser	Val	Thr	Cys	Thr	Leu	Asn	His	Pro	Ser	Leu	Pro	Pro
145					150					155					160
Gln	Arg	Leu	Met	Ala	Leu	Arg	Glu	Pro	Ala	Ala	Gln	Ala	Pro	Val	Lys
				165					170					175	
Leu	Ser	Leu	Asn	Leu	Leu	Ala	Ser	Ser	Asp	Pro	Pro	Glu	Ala	Ala	Ser
			180					185					190		
Trp	Leu	Leu	Cys	Glu	Val	Ser	Gly	Phe	Ser	Pro	Pro	Asn	Ile	Leu	Leu
		195					200					205			
Met	Trp	Leu	Glu	Asp	Gln	Arg	Glu	Val	Asn	Thr	Ser	Gly	Phe	Ala	Pro
	210					215					220				
Ala	Arg	Pro	Pro	Pro	Gln	Pro	Gly	Ser	Thr	Thr	Phe	Trp	Ala	Trp	Ser
225					230					235					240
Val	Leu	Arg	Val	Pro	Ala	Pro	Pro	Ser	Pro	Gln	Pro	Ala	Thr	Tyr	Thr
				245					250					255	
Cys	Val	Val	Ser	His	Glu	Asp	Ser	Arg	Thr	Leu	Leu	Asn	Ala	Ser	Arg
			260					265					270		
Ser	Leu	Glu	Val	Ser	Tyr	Val	Thr	Asp	His						
		275					280								

<210> 155

<211> 335

<212> PRT

<213> Artificial Sequence

<220>

<223> tEGFR

<400> 155

Arg	Lys	Val	Cys	Asn	Gly	Ile	Gly	Ile	Gly	Glu	Phe	Lys	Asp	Ser	Leu
1				5					10					15	
Ser	Ile	Asn	Ala	Thr	Asn	Ile	Lys	His	Phe	Lys	Asn	Cys	Thr	Ser	Ile
			20				25						30		
Ser	Gly	Asp	Leu	His	Ile	Leu	Pro	Val	Ala	Phe	Arg	Gly	Asp	Ser	Phe
		35					40					45			
Thr	His	Thr	Pro	Pro	Leu	Asp	Pro	Gln	Glu	Leu	Asp	Ile	Leu	Lys	Thr
	50					55					60				
Val	Lys	Glu	Ile	Thr	Gly	Phe	Leu	Leu	Ile	Gln	Ala	Trp	Pro	Glu	Asn
65					70				75					80	
Arg	Thr	Asp	Leu	His	Ala	Phe	Glu	Asn	Leu	Glu	Ile	Ile	Arg	Gly	Arg
				85					90					95	
Thr	Lys	Gln	His	Gly	Gln	Phe	Ser	Leu	Ala	Val	Val	Ser	Leu	Asn	Ile
			100					105					110		
Thr	Ser	Leu	Gly	Leu	Arg	Ser	Leu	Lys	Glu	Ile	Ser	Asp	Gly	Asp	Val
		115					120					125			
Ile	Ile	Ser	Gly	Asn	Lys	Asn	Leu	Cys	Tyr	Ala	Asn	Thr	Ile	Asn	Trp
		130				135						140			
Lys	Lys	Leu	Phe	Gly	Thr	Ser	Gly	Gln	Lys	Thr	Lys	Ile	Ile	Ser	Asn
145					150					155					160
Arg	Gly	Glu	Asn	Ser	Cys	Lys	Ala	Thr	Gly	Gln	Val	Cys	His	Ala	Leu
			165						170					175	
Cys	Ser	Pro	Glu	Gly	Cys	Trp	Gly	Pro	Glu	Pro	Arg	Asp	Cys	Val	Ser
			180					185					190		
Cys	Arg	Asn	Val	Ser	Arg	Gly	Arg	Glu	Cys	Val	Asp	Lys	Cys	Asn	Leu
		195					200					205			
Leu	Glu	Gly	Glu	Pro	Arg	Glu	Phe	Val	Glu	Asn	Ser	Glu	Cys	Ile	Gln
	210					215					220				
Cys	His	Pro	Glu	Cys	Leu	Pro	Gln	Ala	Met	Asn	Ile	Thr	Cys	Thr	Gly
225					230					235					240
Arg	Gly	Pro	Asp	Asn	Cys	Ile	Gln	Cys	Ala	His	Tyr	Ile	Asp	Gly	Pro
				245					250					255	
His	Cys	Val	Lys	Thr	Cys	Pro	Ala	Gly	Val	Met	Gly	Glu	Asn	Asn	Thr
			260					265					270		
Leu	Val	Trp	Lys	Tyr	Ala	Asp	Ala	Gly	His	Val	Cys	His	Leu	Cys	His
		275					280					285			
Pro	Asn	Cys	Thr	Tyr	Gly	Cys	Thr	Gly	Pro	Gly	Leu	Glu	Gly	Cys	Pro

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290	295	300	
Thr Asn Gly Pro Lys Ile	Pro Ser Ile Ala Thr	Gly Met Val Gly Ala	
305	310	315	320
Leu Leu Leu Leu Leu Val	Val Ala Leu Gly Ile	Gly Leu Phe Met	
	325	330	335

<210> 156
 <211> 357
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> tEGFR

<400> 156

Met	Leu	Leu	Leu	Val	Thr	Ser	Leu	Leu	Leu	Cys	Glu	Leu	Pro	His	Pro
1				5					10					15	
Ala	Phe	Leu	Leu	Ile	Pro	Arg	Lys	Val	Cys	Asn	Gly	Ile	Gly	Ile	Gly
			20					25					30		
Glu	Phe	Lys	Asp	Ser	Leu	Ser	Ile	Asn	Ala	Thr	Asn	Ile	Lys	His	Phe
		35					40					45			
Lys	Asn	Cys	Thr	Ser	Ile	Ser	Gly	Asp	Leu	His	Ile	Leu	Pro	Val	Ala
		50				55					60				
Phe	Arg	Gly	Asp	Ser	Phe	Thr	His	Thr	Pro	Pro	Leu	Asp	Pro	Gln	Glu
65					70				75					80	
Leu	Asp	Ile	Leu	Lys	Thr	Val	Lys	Glu	Ile	Thr	Gly	Phe	Leu	Leu	Ile
				85					90					95	
Gln	Ala	Trp	Pro	Glu	Asn	Arg	Thr	Asp	Leu	His	Ala	Phe	Glu	Asn	Leu
			100					105					110		
Glu	Ile	Ile	Arg	Gly	Arg	Thr	Lys	Gln	His	Gly	Gln	Phe	Ser	Leu	Ala
		115					120					125			
Val	Val	Ser	Leu	Asn	Ile	Thr	Ser	Leu	Gly	Leu	Arg	Ser	Leu	Lys	Glu
		130				135					140				
Ile	Ser	Asp	Gly	Asp	Val	Ile	Ile	Ser	Gly	Asn	Lys	Asn	Leu	Cys	Tyr
145					150					155				160	
Ala	Asn	Thr	Ile	Asn	Trp	Lys	Lys	Leu	Phe	Gly	Thr	Ser	Gly	Gln	Lys
				165					170					175	
Thr	Lys	Ile	Ile	Ser	Asn	Arg	Gly	Glu	Asn	Ser	Cys	Lys	Ala	Thr	Gly
			180					185					190		
Gln	Val	Cys	His	Ala	Leu	Cys	Ser	Pro	Glu	Gly	Cys	Trp	Gly	Pro	Glu
		195					200					205			
Pro	Arg	Asp	Cys	Val	Ser	Cys	Arg	Asn	Val	Ser	Arg	Gly	Arg	Glu	Cys
		210				215						220			
Val	Asp	Lys	Cys	Asn	Leu	Leu	Glu	Gly	Glu	Pro	Arg	Glu	Phe	Val	Glu
225					230					235				240	
Asn	Ser	Glu	Cys	Ile	Gln	Cys	His	Pro	Glu	Cys	Leu	Pro	Gln	Ala	Met
				245					250					255	
Asn	Ile	Thr	Cys	Thr	Gly	Arg	Gly	Pro	Asp	Asn	Cys	Ile	Gln	Cys	Ala
			260					265					270		
His	Tyr	Ile	Asp	Gly	Pro	His	Cys	Val	Lys	Thr	Cys	Pro	Ala	Gly	Val
		275					280					285			
Met	Gly	Glu	Asn	Asn	Thr	Leu	Val	Trp	Lys	Tyr	Ala	Asp	Ala	Gly	His
290						295					300				
Val	Cys	His	Leu	Cys	His	Pro	Asn	Cys	Thr	Tyr	Gly	Cys	Thr	Gly	Pro
305					310					315					320
Gly	Leu	Glu	Gly	Cys	Pro	Thr	Asn	Gly	Pro	Lys	Ile	Pro	Ser	Ile	Ala
				325					330					335	
Thr	Gly	Met	Val	Gly	Ala	Leu	Leu	Leu	Leu	Leu	Val	Val	Ala	Leu	Gly
			340					345					350		
Ile	Gly	Leu	Phe	Met											
		355													

<210> 157
 <211> 27
 <212> PRT
 <213> Homo sapiens

<220>

<223> CD28 (amino acids 153-179 of Accession No. P10747)

<400> 157

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
 1 5 10 15
 Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
 20 25

<210> 158

<211> 66

<212> PRT

<213> Homo sapiens

<220>

<223> CD28 (amino acids 114-179 of Accession No. P10747)

<400> 158

Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn
 1 5 10 15
 Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu
 20 25 30
 Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly Gly
 35 40 45
 Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe
 50 55 60
 Trp Val
 65

<210> 159

<211> 41

<212> PRT

<213> Homo sapiens

<220>

<223> CD28 (amino acids 180-220 of P10747)

<400> 159

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
 1 5 10 15
 Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
 20 25 30
 Pro Arg Asp Phe Ala Ala Tyr Arg Ser
 35 40

<210> 160

<211> 41

<212> PRT

<213> Homo sapiens

<220>

<223> CD28 (LL to GG)

<400> 160

Arg Ser Lys Arg Ser Arg Gly Gly His Ser Asp Tyr Met Asn Met Thr
 1 5 10 15
 Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
 20 25 30
 Pro Arg Asp Phe Ala Ala Tyr Arg Ser
 35 40

<210> 161

<211> 42

<212> PRT
 <213> Homo sapiens

<220>
 <223> 4-1BB (amino acids 214-255 of Q07011.1)

<400> 161
 Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
 1 5 10 15
 Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
 20 25 30
 Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
 35 40

<210> 162
 <211> 112
 <212> PRT
 <213> Homo sapiens

<220>
 <223> CD3 zeta

<400> 162
 Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
 1 5 10 15
 Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
 20 25 30
 Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
 35 40 45
 Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
 50 55 60
 Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
 65 70 75 80
 Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
 85 90 95
 Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
 100 105 110

<210> 163
 <211> 112
 <212> PRT
 <213> Homo sapiens

<220>
 <223> CD3 zeta

<400> 163
 Arg Val Lys Phe Ser Arg Ser Ala Glu Pro Pro Ala Tyr Gln Gln Gly
 1 5 10 15
 Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
 20 25 30
 Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
 35 40 45
 Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
 50 55 60
 Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
 65 70 75 80
 Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
 85 90 95
 Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
 100 105 110

<210> 164
 <211> 112
 <212> PRT

<213> Homo sapiens

<220>

<223> CD3 zeta

<400> 164

Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly
1				5				10						15	
Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr
			20					25					30		
Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys
		35					40					45			
Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys
	50					55					60				
Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg
65					70				75					80	
Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala
			85					90						95	
Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg
			100					105					110		